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(54) Title: VARIABLE HEAVY CHAIN AND VARIABLE LIGHT CHAIN REGIONS OF ANTIBODIES TO HUMAN
PLATELET GLYCOPROTEIN IB ALPHA

(57) Abstract: The present invention is directed to a method of selecting a clone that binds to human platelet glycoprotein Ib alpha using a human variable heavy chain and variable light chain immunoglobulin library. The invention is further directed to isolated nucleic acid molecules encoding a variable heavy chain or variable light chain region of an antibody, wherein the antibody binds to human platelet glycoprotein Ib alpha and inhibits aggregation of platelets. Expression vectors and host cells comprising the nucleic acid molecules are also provided, as well as methods for producing the variable heavy chain or the variable light chain region. An isolated variable heavy chain or variable light chain region of an antibody, wherein the antibody binds to human platelet glycoprotein Ib alpha and inhibits aggregation of platelets, is also provided. An antibody comprising the variable heavy chain or variable light chain regions is provided, as is a composition comprising the antibody and a carrier. The subject invention further provides a method of inhibiting aggregation of platelets, as well as a method of binding human platelet glycoprotein Ib alpha. A method of selecting a variable heavy chain or variable light chain region of an antibody is also provided.

VARIABLE HEAVY CHAIN AND VARIABLE LIGHT CHAIN REGIONS OF
ANTIBODIES TO HUMAN PLATELET GLYCOPROTEIN IB ALPHA

This application claims priority of U.S. Provisional
5 Patent Application No. 60/106,275, filed October 30,
1998.

FIELD OF THE INVENTION

The subject invention is directed generally to human
10 platelet glycoprotein Ib alpha, and more particularly to
variable heavy chain and variable light chain regions of
antibodies to human platelet glycoprotein Ib alpha and
uses thereof.

15 BACKGROUND OF THE INVENTION

Throughout this application various publications are
referenced, many in parenthesis. Full citations for each
of these publications are provided at the end of the
Detailed Description. The disclosures of each of these
20 publications in their entireties are hereby incorporated
by reference in this application.

The platelet glycoprotein Ib/IX (GPIb/IX) receptor
for von Willebrand factor (vWf) is believed to consist of
a 1:1 heterodimeric complex (Du et al. 1987) between GPIb
25 (160 kDa) and GPIX (17 kDa) in a noncovalent association.
GPIb in turn consists of a disulfide-linked 140 kDa alpha
chain (GPIb alpha) and a 22 kDa beta chain (GPIb beta)
(Fitzgerald and Phillips 1989).

The GPIb/IX complex comprises one of the major
30 transmembrane receptor complexes on blood platelets (Roth
1991; Lopez 1994; Clemetson and Clemetson 1995),
mediating von Willebrand factor (vWF)-dependent platelet
adhesion. In the 1980's, Miller et al. developed a
series of monoclonal antibodies (mab) directed against
35 the GP Ib/IX complex receptor for vWf. In particular,
monoclonal antibody C-34 was characterized in detail and
it was determined that mab C-34 recognized an epitope

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within the platelet glycoprotein Ib/IX complex (Miller et al. 1990). In this and subsequent work, Miller et al. showed that monoclonal antibodies C-34, AS-2 and AS-7 were potent inhibitors of the ristocetin-induced
5 aggregation of normal platelets that was dependent upon von Willebrand factor. Miller et al. also showed that the epitopes for all three monoclonal antibodies lay within the GPIb/IX complex.

Attempts to define the binding sites for various
10 monoclonal antibodies have led to the development of epitope libraries. Parmley and Smith developed a bacteriophage expression vector that could display foreign epitopes on its surface (Parmley and Smith 1988). This vector could be used to construct large collections
15 of bacteriophage which could include virtually all possible sequences of a short (e.g. six-amino-acid) peptide. They also developed biopanning, which is a method for affinity-purifying phage displaying foreign epitopes using a specific antibody (see Parmley and Smith
20 1988; Cwirla et al. 1990; Scott and Smith 1990; Christian et al. 1992; Smith and Scott 1993).

After the development of epitope libraries, Smith et al. then suggested that it should be possible to use the bacteriophage expression vector and biopanning technique
25 of Parmley and Smith to identify epitopes from all possible sequences of a given length. This led to the idea of identifying peptide ligands for antibodies by biopanning epitope libraries, which could then be used in vaccine design, epitope mapping, the identification of
30 genes, and many other applications (Parmley and Smith 1988; Scott 1992).

Antibody fragments have also been displayed on the surface of filamentous phage that encode the antibody genes (Hoogenboom and Winter 1992; McCafferty et al.

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1990; Vaughan et al. 1996; Tomlinson et al. 1992; Nissim et al. 1994; Griffiths et al. 1994). Variable heavy chain (V_H) and variable light chain (V_L) immunoglobulin libraries have thus been developed in phage, and phage
5 can be selected by panning with antibody. The encoded antibody fragments can then be secreted as soluble fragments from infected bacteria. This display of antibodies on phage and selection with antigen mimics immune selection and can be used to make antibodies
10 without immunization from a single library of phage (see Hoogenboom and Winter 1992).

A human synthetic V_H and V_L ScFv library was made by recloning the heavy and light chain variable regions from the lox library vectors (Griffiths et al. 1994) into the
15 phagemid vector pHEN2 (see Fig. 1). This "Griffin.1" library is a ScFv phagemid library made from synthetic V-gene segments. The World Wide Web address to download the germline V gene sequences which comprise the Griffin.1 library is [http://www.mrc-cpe.cam.ac.uk/](http://www.mrc-cpe.cam.ac.uk/imt-doc/vbase-questions.html)
20 [imt-doc/vbase-questions.html](http://www.mrc-cpe.cam.ac.uk/imt-doc/vbase-questions.html).

A need continues to exist for the elucidation of the sequence of useful epitopes of antibodies that bind to glycoprotein Ib alpha.

25

SUMMARY OF THE INVENTION

To this end, the subject invention provides a method of selecting a clone that binds to human platelet glycoprotein Ib alpha using a human variable heavy chain and variable light chain immunoglobulin library. The
30 method comprises: incubating a human variable heavy chain and variable light chain immunoglobulin library with cells expressing human platelet glycoprotein Ib, and selecting clones of the library which bind to the cells; and incubating the selected clones of the library with

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washed human platelets, and selecting resulting clones which bind to the washed human platelets, wherein the resulting clones bind to human platelet glycoprotein Ib alpha.

5 The subject invention further provides an isolated nucleic acid molecule encoding a variable heavy chain or a variable light chain region of an antibody, wherein the antibody binds to human platelet glycoprotein Ib alpha and inhibits aggregation of platelets.

10 The isolated nucleic acid molecules of the invention can be inserted into suitable expression vectors and/or host cells. Expression of the nucleic acid molecules encoding a variable heavy chain or a variable light chain region results in production of variable heavy chain or
15 variable light chain regions of an antibody (wherein the antibody binds to human platelet glycoprotein Ib alpha and inhibits aggregation of platelets) in a host cell.

Further provided is an isolated nucleic acid molecule encoding a variable heavy chain region of an
20 antibody, wherein the antibody binds to human platelet glycoprotein Ib alpha and inhibits aggregation of platelets, the nucleic acid molecule encoding a first amino acid sequence having at least 90% amino acid identity to a second amino acid sequence. The second
25 amino acid sequence is selected from the group consisting of SEQ ID NOS:10-15.

Also provided is an isolated nucleic acid molecule encoding a variable light chain region of an antibody, wherein the antibody binds to human platelet glycoprotein
30 Ib alpha and inhibits aggregation of platelets, the nucleic acid molecule encoding a first amino acid sequence having at least 90% amino acid identity to a second amino acid sequence. The second amino acid

sequence is selected from the group consisting of SEQ ID NOs:16-21.

The invention also provides an isolated variable heavy chain or a variable light chain region of an antibody, wherein the antibody binds to human platelet glycoprotein Ib alpha and inhibits aggregation of platelets. Further provided is an isolated variable heavy chain region of an antibody, wherein the antibody binds to human platelet glycoprotein Ib alpha and inhibits aggregation of platelets, the variable heavy chain region having a first amino acid sequence having at least 90% amino acid identity to a second amino acid sequence, the second amino acid sequence selected from the group consisting of SEQ ID NOs:10-15. Also provided is an isolated variable light chain region of an antibody, wherein the antibody binds to human platelet glycoprotein Ib alpha and inhibits aggregation of platelets, the variable light chain region having a first amino acid sequence having at least 90% amino acid identity to a second amino acid sequence, the second amino acid sequence selected from the group consisting of SEQ ID NOs:16-21.

Further provided is an antibody comprising the variable heavy chain or variable light chain region of the subject invention, as well as a composition comprising the antibody. The subject invention further provides a method of inhibiting aggregation of platelets by exposing platelets to the composition. Further provided is a method of binding human platelet glycoprotein Ib alpha by exposing human platelet glycoprotein Ib alpha to the antibody.

Also provided is a method of selecting a variable heavy chain or variable light chain region of an

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antibody, wherein the antibody inhibits aggregation of platelets. The method comprises: selecting a variable heavy chain or variable light chain region according to the subject invention, wherein each of the variable heavy chain or variable light chain regions has an amino acid sequence; altering the amino acid sequence of the selected variable heavy chain or variable light chain region; constructing an antibody having the altered amino acid sequence of the variable heavy chain or variable light chain region; and determining whether the antibody inhibits aggregation of platelets, wherein the altered variable heavy chain or variable light chain region of an antibody that inhibits aggregation of platelets is thereby selected.

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BRIEF DESCRIPTION OF THE DRAWINGS

These and other features and advantages of this invention will be evident from the following detailed description of preferred embodiments when read in conjunction with the accompanying drawings in which:

20

Fig. 1 is a map of the pHEN2 phagemid vector;

Fig. 2 illustrates the structure of an antibody;

Fig. 3 illustrates the structure of the Fab-fragment of an antibody;

25

Fig. 4 illustrates the structure of the Fv-fragment of an antibody;

Fig. 5 illustrates the amino acid sequence with alignment for H1b-1;

30

Fig. 6 illustrates the amino acid sequence with alignment for H1b-2;

Fig. 7 illustrates the amino acid sequence with alignment for H1b-3;

Fig. 8 illustrates the amino acid sequence with alignment for H1b-5;

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Fig. 9 illustrates the amino acid sequence with alignment for H1b-6;

Fig. 10 is a direct western blot of H1b-1 human anti-GPIb alpha;

5 Fig. 11 shows western blots of H1b-1, H1b-2 and SZ-2 under non-reduced and reduced conditions;

Fig. 12 illustrates inhibition of ristocetin-induced platelet aggregation by clonal phagemid expressing human VH and VL ScFv;

10 Fig. 13 illustrates inhibition of botrocetin-induced aggregation of formalin-fixed human platelets; and

Fig. 14 illustrates inhibition of antibody by PRP aggregation induced by ristocetin.

15 **DETAILED DESCRIPTION OF THE INVENTION**

As used herein, antibody, variable heavy chain (V_H or V_h) and variable light chain (V_L or V_l), Fv fragment, and CDR (hypervariable regions) (CDR1, CDR2, CDR3), are used in the context of Figs. 2-4. Fig. 1 shows a schematic drawing of the organization of a natural IgG and derived recombina

20 nt fragments. The Fv fragment is shown as a "Single Chain Fv Fragment" (ScFv) in Fig. 4. In this type of recombinant protein, the two antigen binding regions of the light and heavy chain (V_h and V_l) are

25 connected by a 15-18 amino acid peptide. This linker region permits appropriate interaction between the V_h and V_l regions. A further description of such recombinant antibody structure can be found at <http://www.mgen.uni-heidelberg.de/SD/SDscFvSite.html>.

30 The term "nucleic acid", as used herein, refers to either DNA or RNA. "Nucleic acid sequence" or "polynucleotide sequence" refers to a single- or double-stranded polymer of deoxyribonucleotide or ribonucleotide bases read from the 5' to the 3' end. It

includes both self-replicating plasmids, infectious polymers of DNA or RNA, and nonfunctional DNA or RNA.

"Isolated" nucleic acid refers to nucleic acid which has been separated from an organism in a substantially purified form (i.e. substantially free of other substances originating from that organism), and to synthetic nucleic acid.

By a nucleic acid sequence "homologous to" or "complementary to", it is meant a nucleic acid that selectively hybridizes, duplexes or binds to DNA sequences encoding the variable heavy (V_H) or variable light (V_L) chain or portions thereof when the DNA sequences encoding the variable heavy (V_H) or variable light (V_L) chain are present in a human genomic or cDNA library.

A DNA sequence which is similar or complementary to a target sequence can include sequences which are shorter or longer than the target sequence so long as they meet the functional test set forth.

Typically, the hybridization is done in a Southern blot protocol using a 0.2X SSC, 0.1% SDS, 65°C wash. The term "SSC" refers to a citrate-saline solution of 0.15M sodium chloride and 20 mM sodium citrate. Solutions are often expressed as multiples or fractions of this concentration. For example, 6X SSC refers to a solution having a sodium chloride and sodium citrate concentration of 6 times this amount or 0.9 M sodium chloride and 120 mM sodium citrate. 0.2X SSC refers to a solution 0.2 times the SSC concentration or 0.03M sodium chloride and 4 mM sodium citrate.

The phrase "nucleic acid molecule encoding" refers to a nucleic acid molecule which directs the expression of a specific protein or peptide (in this case, a variable heavy (V_H) or variable light (V_L) chain). The nucleic acid sequences include both the DNA strand

sequence that is transcribed into RNA and the RNA
sequence that is translated into protein or peptide (or
variable heavy (V_H) or variable light (V_L) chain). The
nucleic acid molecule includes both the full length
5 nucleic acid sequences as well as non-full length
sequences derived from the full length variable heavy (V_H)
or variable light (V_L) chain. It being further understood
that the sequence includes the degenerate codons of the
native sequence or sequences which may be introduced to
10 provide codon preference in a specific host cell.

The term "located upstream" as used herein refers to
linkage of a promoter upstream from a nucleic acid (DNA)
sequence such that the promoter mediates transcription of
the nucleic acid (DNA) sequence.

15 The term "vector", refers to viral expression
systems, autonomous self-replicating circular DNA
(plasmids), phagemids, and includes both expression and
nonexpression plasmids and phagemids. Where a
recombinant microorganism or cell is described as hosting
20 an "expression vector," this includes both
extrachromosomal circular DNA and DNA that has been
incorporated into the host chromosome(s). Where a vector
is being maintained by a host cell, the vector may either
be stably replicated by the cells during mitosis as an
25 autonomous structure, or the vector may be incorporated
within the host's genome.

The term "plasmid" refers to an autonomous circular
DNA molecule capable of replication in a cell, and
includes both the expression and nonexpression types.
30 Where a recombinant microorganism or cell is described as
hosting an "expression plasmid", this includes latent
viral DNA integrated into the host chromosome(s). Where
a plasmid is being maintained by a host cell, the plasmid
is either being stably replicated by the cell during

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mitosis as an autonomous structure, or the plasmid is incorporated within the host's genome.

The term "phagemid" refers to a vector which combines attributes of a bacteriophage and a plasmid.

5 The phrase "heterologous protein" or "recombinantly produced heterologous protein" refers to a peptide or protein of interest (in this case the variable heavy (V_H) or variable light (V_L) chain) produced using cells that do not have an endogenous copy of DNA able to express the
10 peptide or protein of interest. The cells produce the peptide or protein because they have been genetically altered by the introduction of the appropriate nucleic acid sequences. The recombinant peptide or protein will not be found in association with peptides or proteins and
15 other subcellular components normally associated with the cells producing the peptide or protein.

The following terms are used to describe the sequence relationships between two or more nucleic acid molecules or polynucleotides, or between two or more
20 amino acid sequences of peptides or proteins (in this case, the variable heavy (V_H) or variable light (V_L) chain): "reference sequence", "comparison window", "sequence identity", "sequence homology", "percentage of sequence identity", "percentage of sequence homology",
25 "substantial identity", and "substantial homology". A "reference sequence" is a defined sequence used as a basis for a sequence comparison; a reference sequence may be a subset of a larger sequence, for example, as a segment of a full-length cDNA or gene sequence given in a
30 sequence listing or may comprise a complete cDNA or gene sequence.

Optimal alignment of sequences for aligning a comparison window may be conducted, for example, by the local homology algorithm of Smith and Waterman (1981), by

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the homology alignment algorithm of Needleman and Wunsch (1970), by the search for similarity method of Pearson and Lipman (1988), or by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the 5 Wisconsin Genetics Software Package Release 7.0, Genetics Computer Group, 575 Science Dr., Madison, Wis.).

As applied to nucleic acid molecules or polynucleotides, the terms "substantial identity" or "substantial sequence identity" mean that two nucleic 10 acid sequences, when optimally aligned (see above), share at least 90 percent sequence identity, preferably at least 95 percent sequence identity, more preferably at least 96, 97, 98 or 99 percent sequence identity.

"Percentage nucleotide (or nucleic acid) identity" 15 or "percentage nucleotide (or nucleic acid) sequence identity" refers to a comparison of the nucleotides of two nucleic acid molecules which, when optimally aligned, have approximately the designated percentage of the same nucleotides. For example, "95% nucleotide identity" 20 refers to a comparison of the nucleotides of two nucleic acid molecules which when optimally aligned have 95% nucleotide identity. Preferably, nucleotide positions which are not identical differ by redundant nucleotide substitutions (the nucleotide substitution does not 25 change the amino acid encoded by the particular codon).

As further applied to nucleic acid molecules or polynucleotides, the terms "substantial homology" or "substantial sequence homology" mean that two nucleic acid sequences, when optimally aligned (see above), share 30 at least 90 percent sequence homology, preferably at least 95 percent sequence homology, more preferably at least 96, 97, 98 or 99 percent sequence homology.

"Percentage nucleotide (or nucleic acid) homology" or "percentage nucleotide (or nucleic acid) sequence

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homology" refers to a comparison of the nucleotides of two nucleic acid molecules which, when optimally aligned, have approximately the designated percentage of the same nucleotides or nucleotides which are not identical but
5 differ by redundant nucleotide substitutions (the nucleotide substitution does not change the amino acid encoded by the particular codon). For example, "95% nucleotide homology" refers to a comparison of the nucleotides of two nucleic acid molecules which when
10 optimally aligned have 95% nucleotide homology.

As applied to polypeptides, the terms "substantial identity" or "substantial sequence identity" mean that two peptide sequences, when optimally aligned, such as by the programs GAP or BESTFIT using default gap, share at
15 least 90 percent sequence identity, preferably at least 95 percent sequence identity, more preferably at least 96, 97, 98 or 99 percent sequence identity.

"Percentage amino acid identity" or "percentage amino acid sequence identity" refers to a comparison of
20 the amino acids of two polypeptides which, when optimally aligned, have approximately the designated percentage of the same amino acids. For example, "95% amino acid identity" refers to a comparison of the amino acids of two polypeptides which when optimally aligned have 95%
25 amino acid identity. Preferably, residue positions which are not identical differ by conservative amino acid substitutions. For example, the substitution of amino acids having similar chemical properties such as charge or polarity are not likely to affect the properties of a
30 protein. Examples include glutamine for asparagine or glutamic acid for aspartic acid.

As further applied to polypeptides, the terms "substantial homology" or "substantial sequence homology" mean that two peptide sequences, when optimally aligned,

such as by the programs GAP or BESTFIT using default gap, share at least 90 percent sequence homology, preferably at least 95 percent sequence homology, more preferably at least 96, 97, 98 or 99 percent sequence homology.

5 "Percentage amino acid homology" or "percentage amino acid sequence homology" refers to a comparison of the amino acids of two polypeptides which, when optimally aligned, have approximately the designated percentage of the same amino acids or conservatively substituted amino
10 acids. For example, "95% amino acid homology" refers to a comparison of the amino acids of two polypeptides which when optimally aligned have 95% amino acid homology. As used herein, homology refers to identical amino acids or residue positions which are not identical but differ only
15 by conservative amino acid substitutions. For example, the substitution of amino acids having similar chemical properties such as charge or polarity are not likely to affect the properties of a protein. Examples include glutamine for asparagine or glutamic acid for aspartic
20 acid.

The phrase "substantially purified" or "isolated" when referring to a protein (or peptide), means a chemical composition which is essentially free of other cellular components. It is preferably in a homogeneous
25 state although it can be in either a dry or aqueous solution. Purity and homogeneity are typically determined using analytical chemistry techniques such as polyacrylamide gel electrophoresis or high performance liquid chromatography. A protein (or peptide) which is
30 the predominant species present in a preparation is substantially purified. Generally, a substantially purified or isolated protein (or peptide) will comprise more than 80% of all macromolecular species present in the preparation. Preferably, the protein (or peptide) is

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purified to represent greater than 90% of all macromolecular species present. More preferably the protein (or peptide) is purified to greater than 95%, and most preferably the protein (or peptide) is purified to essential homogeneity, wherein other macromolecular species are not detected by conventional techniques. A "substantially purified" or "isolated" protein (or peptide) can be separated from an organism, synthetically or chemically produced, or recombinantly produced.

10 "Biological sample" or "sample" as used herein refers to any sample obtained from a living organism or from an organism that has died. Examples of biological samples include body fluids and tissue specimens.

High stringent hybridization conditions are selected at about 5°C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength and pH. The T_m is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe. Typically, stringent conditions will be those in which the salt concentration is at least about 0.02 molar at pH 7 and the temperature is at least about 60°C. As other factors may significantly affect the stringency of hybridization, including, among others, base composition and size of the complementary strands, the presence of organic solvents, ie. salt or formamide concentration, and the extent of base mismatching, the combination of parameters is more important than the absolute measure of any one. High stringency may be attained, for example, by overnight hybridization at about 68°C in a 6X SSC solution, washing at room temperature with 6X SSC solution, followed by washing at about 68°C in a 6X SSC solution then in a 0.6X SSX solution.

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Hybridization with moderate stringency may be attained, for example, by: 1) filter pre-hybridizing and hybridizing with a solution of 3X sodium chloride, sodium citrate (SSC), 50% formamide, 0.1M Tris buffer at pH 7.5, 5 5X Denhardt's solution; 2) pre-hybridization at 37°C for 4 hours; 3) hybridization at 37°C with amount of labelled probe equal to 3,000,000 cpm total for 16 hours; 4) wash in 2X SSC and 0.1% SDS solution; 5) wash 4X for 1 minute each at room temperature and 4X at 60°C for 30 minutes 10 each; and 6) dry and expose to film.

The phrase "selectively hybridizing to" refers to a nucleic acid molecule that hybridizes, duplexes or binds only to a particular target DNA or RNA sequence when the target sequences are present in a preparation of total 15 cellular DNA or RNA. By selectively hybridizing it is meant that a nucleic acid molecule binds to a given target in a manner that is detectable in a different manner from non-target sequence under moderate, or more preferably under high, stringency conditions of 20 hybridization. "Complementary" or "target" nucleic acid sequences refer to those nucleic acid sequences which selectively hybridize to a nucleic acid molecule. Proper annealing conditions depend, for example, upon a nucleic acid molecule's length, base composition, and the number 25 of mismatches and their position on the molecule, and must often be determined empirically. For discussions of nucleic acid molecule (probe) design and annealing conditions, see, for example, Sambrook et al. 1989.

It will be readily understood by those skilled in 30 the art and it is intended here, that when reference is made to particular sequence listings, such reference includes sequences which substantially correspond to its complementary sequence and those described including allowances for minor sequencing errors, single base

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changes, deletions, substitutions and the like, such that any such sequence variation corresponds to the nucleic acid sequence of the peptide/protein to which the relevant sequence listing relates.

5 The DNA molecules of the subject invention also include DNA molecules coding for protein analogs, fragments or derivatives of the protein which differ from naturally-occurring forms (the naturally-occurring protein) in terms of the identity or location of one or
10 more amino acid residues (deletion analogs containing less than all of the residues specified for the protein, substitution analogs wherein one or more residues specified are replaced by other residues, and addition
15 analogs wherein one or more amino acid residues is added to a terminal or medial portion of the protein) and which share the functional property of the naturally-occurring form. These molecules include: the incorporation of codons "preferred" for expression by selected non-mammalian hosts; the provision of sites for cleavage
20 by restriction endonuclease enzymes; and the provision of additional initial, terminal or intermediate DNA sequences that facilitate construction of readily expressed vectors.

As used herein, a "peptide" refers to an amino acid
25 sequence of three to one hundred amino acids, and therefore an isolated peptide that comprises an amino acid sequence is not intended to cover amino acid sequences of greater than 125 amino acids. Proteins and peptides can contain any naturally-occurring or
30 non-naturally-occurring amino acids, including the D-form of the amino acids, amino acid derivatives and amino acid mimics, so long as the desired function and activity of the protein or peptide is maintained. The choice of including an (L)- or a (D)-amino acid in the proteins or

peptides depends, in part, on the desired characteristics of the protein or peptide. For example, the incorporation of one or more (D)-amino acids can confer increased stability on the protein or peptide and can
5 allow a protein or peptide to remain active in the body for an extended period of time. The incorporation of one or more (D)-amino acids can also increase or decrease the pharmacological activity of the protein or peptide.

The proteins or peptides may also be cyclized, since
10 cyclization may provide the proteins or peptides with superior properties over their linear counterparts.

As used herein, the terms "amino acid mimic" and "mimetic" mean an amino acid analog or non-amino acid moiety that has the same or similar functional
15 characteristic of a given amino acid. For instance, an amino acid mimic of a hydrophobic amino acid is one which is non-polar and retains hydrophobicity, generally by way of containing an aliphatic chemical group. By way of further example, an arginine mimic can be an analog of
20 arginine which contains a side chain having a positive charge at physiological pH, as is characteristic of the guanidinium side chain reactive group of arginine.

In addition, modifications to the peptide backbone and peptide bonds thereof are also encompassed within the
25 scope of amino acid mimic or mimetic. Such modifications can be made to the amino acid, derivative thereof, non-amino acid moiety or the peptide either before or after the amino acid, derivative thereof or non-amino acid moiety is incorporated into the peptide. What is
30 critical is that such modifications mimic the peptide backbone and bonds which make up the same and have substantially the same spacial arrangement and distance as is typical for traditional peptide bonds and backbones. An example of one such modification is the

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reduction of the carbonyl(s) of the amide peptide backbone to an amine. A number of reagents are available and well known for the reduction of amides to amines such as those disclosed in Wann et al., JOC, 46:257 (1981) and 5 Raucher et al., Tetrahedron. Lett., 21:14061 (1980). An amino acid mimic is, therefore, an organic molecule that retains the similar amino acid pharmacophore groups as is present in the corresponding amino acid and which exhibits substantially the same spatial arrangement 10 between functional groups.

The substitution of amino acids by non-naturally occurring amino acids and amino acid mimics as described above can enhance the overall activity or properties of an individual protein or peptide based on the 15 modifications to the backbone or side chain functionalities. For example, these types of alterations to the specifically described amino acid substituents can enhance the protein's or peptide's stability to enzymatic breakdown and increase biological activity.

20 Modifications to the peptide backbone similarly can add stability and enhance activity.

One skilled in the art, using the above sequences or formulae, can easily synthesize the proteins or peptides. Standard procedures for preparing synthetic peptides are 25 well known in the art. Peptides can be synthesized using: the solid phase peptide synthesis (SPPS) method of Merrifield (J. Am. Chem. Soc., 85:2149 (1964)) or modifications of SPPS; or, peptides can be synthesized using standard solution methods well known in the art 30 (see, for example, Bodanzsky, M., Principles of Peptide Synthesis, 2nd revised ed., Springer-Verlag (1988 and 1993)). Alternatively, simultaneous multiple peptide synthesis (SMPS) techniques well known in the art can be used. Peptides prepared by the method of Merrifield can

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be synthesized using an automated peptide synthesizer such as the Applied Biosystems 431A-01 Peptide Synthesizer (Mountain View, Calif.) or using the manual peptide synthesis technique described by Houghten, Proc. Natl. Acad. Sci., USA 82:5131 (1985).

With these definitions in mind, the subject invention provides a method of selecting a clone that binds to human platelet glycoprotein Ib alpha using a human variable heavy chain and variable light chain immunoglobulin library. The method comprises: incubating a human variable heavy chain and variable light chain immunoglobulin library with cells expressing human platelet glycoprotein Ib, and selecting clones of the library which bind to the cells; and incubating the selected clones of the library with washed human platelets, and selecting resulting clones which bind to the washed human platelets, wherein the resulting clones bind to human platelet glycoprotein Ib alpha.

Preferably, the cells which express the human platelet glycoprotein Ib alpha are Chinese Hamster Ovary cells.

In one embodiment, the method further comprises incubating the selected resulting clones with further platelets and adding an anti-glycoprotein Ib alpha molecule that may displace clones already bound to the further platelets, and selecting the then-resulting clones that are not bound to the further platelets, the then-resulting clones being capable of binding to human platelet glycoprotein Ib alpha. Preferably, the anti-glycoprotein Ib alpha molecule is a murine monoclonal antibody or peptide (such as the murine monoclonal antibody C-34 or the peptide having the amino acid sequence shown in SEQ ID NO:1).

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The subject invention further provides an isolated nucleic acid molecule encoding a variable heavy chain or a variable light chain region of an antibody, wherein the antibody binds to human platelet glycoprotein Ib alpha
5 and inhibits aggregation of platelets. The nucleic acid molecule can be deoxyribonucleic acid (DNA) or ribonucleic acid (RNA, including messenger RNA or mRNA), genomic or recombinant, biologically isolated or synthetic.

10 The DNA molecule can be a cDNA molecule, which is a DNA copy of a messenger RNA (mRNA) encoding the variable heavy (V_H) or variable light (V_L) chain.

An example of such a variable heavy chain region of an antibody is the variable heavy chain having a
15 nucleotide sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 3, and SEQ ID NO: 4. An example of such a variable light chain region of an antibody is the variable light chain having a nucleotide sequence selected from the group consisting of SEQ ID NOs:5-9.

20 The amino acid sequence encoded by these nucleotide sequences are shown in SEQ ID NOs:10-15 (heavy chains), and SEQ ID NOs:16-21 (light chains).

The nucleic acid molecules of the subject invention can be expressed in suitable recombinant host cells using
25 conventional techniques. Any suitable host and/or vector system can be used to express the variable heavy chain or variable light chain region of an antibody, wherein the antibody binds to human platelet glycoprotein Ib alpha and inhibits aggregation of platelets. For in vitro
30 expression, CHO cells or other mammalian cells, or *Escherichia coli* are preferred.

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Techniques for introducing the nucleic acid molecules into the host cells may involve the use of expression vectors which comprise the nucleic acid molecules. These expression vectors (such as phagemids, 5 plasmids, and viruses; viruses including bacteriophage) can then be used to introduce the nucleic acid molecules into suitable host cells. For example, DNA encoding the variable heavy chain or variable light chain region of an antibody can be injected into the nucleus of a host cell 10 or transformed into the host cell using a suitable vector, or mRNA encoding the variable heavy chain or variable light chain region can be injected directly into the host cell, in order to obtain expression of variable heavy chain or variable light chain regions of an 15 antibody in the host cell.

Various methods are known in the art for introducing nucleic acid molecules into host cells. One method is microinjection, in which DNA is injected directly into the nucleus of cells through fine glass needles (or RNA 20 is injected directly into the cytoplasm of cells). Alternatively, DNA can be incubated with an inert carbohydrate polymer (dextran) to which a positively charged chemical group (DEAE, for diethylaminoethyl) has been coupled. The DNA sticks to the DEAE-dextran via its 25 negatively charged phosphate groups. These large DNA-containing particles stick in turn to the surfaces of cells, which are thought to take them in by a process known as endocytosis. Some of the DNA evades destruction in the cytoplasm of the cell and escapes to the nucleus, 30 where it can be transcribed into RNA like any other gene in the cell. In another method, cells efficiently take in DNA in the form of a precipitate with calcium phosphate. In electroporation, cells are placed in a solution containing DNA and subjected to a brief

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electrical pulse that causes holes to open transiently in their membranes. DNA enters through the holes directly into the cytoplasm, bypassing the endocytotic vesicles through which they pass in the DEAE-dextran and calcium phosphate procedures. DNA can also be incorporated into artificial lipid vesicles, liposomes, which fuse with the cell membrane, delivering their contents directly into the cytoplasm. In an even more direct approach, DNA is absorbed to the surface of tungsten microprojectiles and fired into cells with a device resembling a shotgun.

Several of these methods, microinjection, electroporation, and liposome fusion, have been adapted to introduce proteins into cells. For review, see Mannino and Gould-Fogerite 1988, Shigekawa and Dower 1988, Capecchi 1980, and Klein et al. 1987.

Further methods for introducing nucleic acid molecules into cells involve the use of viral vectors. One such virus widely used for protein production is an insect virus, baculovirus. For a review of baculovirus vectors, see Miller (1989). Various viral vectors have also been used to transform mammalian cells, such as bacteriophage, vaccinia virus, adenovirus, and retrovirus.

As indicated, some of these methods of transforming a cell require the use of an intermediate plasmid vector. U.S. Patent No. 4,237,224 to Cohen and Boyer describes the production of expression systems in the form of recombinant plasmids using restriction enzyme cleavage and ligation with DNA ligase. These recombinant plasmids are then introduced by means of transformation and replicated in unicellular cultures including procaryotic organisms and eucaryotic cells grown in tissue culture. The DNA sequences are cloned into the plasmid vector

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using standard cloning procedures known in the art, as described by Sambrook et al. (1989).

It should be readily apparent that several of these methods can be used to introduce the nucleic acid molecules into the cells of, or implants of cells within, a subject in vivo (gene therapy applications, including human gene therapy). For example, nucleic acid encoding the variable heavy chain and/or variable light chain, or encoding fragments thereof, or encoding an antibody comprising the variable heavy chain and/or variable light chain or fragments thereof, could be introduced in vivo using a mammalian viral vector such as adenovirus. Such a vector could also include and introduce an inducible promoter controlling expression of the nucleic acid, or other suitable positive or negative response element, so that the subject could simply take a "drug" that would turn on or turn off the expression of the nucleic acid of the subject invention. The "drug", for example, could induce the inducible promoter.

Host cells into which the nucleic acid encoding the variable heavy chain or variable light chain region has been introduced can be used to produce (i.e. to functionally express) the variable heavy chain or variable light chain region. The function of the encoded variable heavy chain or a variable light chain region can be assayed according to methods known in the art by incorporating the variable heavy chain or variable light chain region into an antibody, and testing the antibody for its ability to bind to human platelet glycoprotein Ib alpha and to inhibit aggregation of platelets.

The nucleic acid molecules of the subject invention can be used either as probes or for the design of primers to obtain DNA encoding other variable heavy chain or variable light chain regions of an antibody, wherein the

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antibody binds to human platelet glycoprotein Ib alpha and inhibits aggregation of platelet, by either cloning and colony/plaque hybridization or amplification using the polymerase chain reaction (PCR).

5 Specific probes derived from the sequences herein can be employed to identify colonies or plaques containing cloned DNA encoding a variable heavy chain or variable light chain region of an antibody using known methods (see Sambrook et al. 1989). One skilled in the
10 art will recognize that by employing such probes under high stringency conditions (for example, hybridization at 42°C with 5X SSPC and 50% formamide, washing at 50-65°C with 0.5X SSPC), sequences having regions which are greater than 90% homologous or identical to the probe can
15 be obtained. Sequences with lower percent homology or identity to the probe, which also encode variable heavy chain or variable light chain regions of an antibody, can be obtained by lowering the stringency of hybridization and washing (e.g., by reducing the hybridization and wash
20 temperatures or reducing the amount of formamide employed).

Specific primers derived from the sequences herein can be used in PCR to amplify a DNA sequence encoding a variable heavy chain or variable light chain region of an
25 antibody, wherein the antibody binds to human platelet glycoprotein Ib alpha and inhibits aggregation of platelets, using known methods (see Innis et al. 1990). One skilled in the art will recognize that by employing such primers under high stringency conditions (for
30 example, annealing at 50-60°C, depending on the length and specific nucleotide content of the primers employed), sequences having regions greater than 75% homologous or identical to the primers will be amplified.

Various modifications of the nucleic acid and amino acid sequences disclosed herein are covered by the subject invention. These varied sequences still encode a functional variable heavy chain or variable light chain

5 region of an antibody, wherein the antibody binds to human platelet glycoprotein Ib alpha and inhibits aggregation of platelets. The invention thus further provides an isolated nucleic acid molecule encoding a variable heavy chain region of an antibody, wherein the

10 antibody binds to human platelet glycoprotein Ib alpha and inhibits aggregation of platelets, the nucleic acid molecule encoding a first amino acid sequence having at least 90% amino acid identity to a second amino acid sequence, the second amino acid sequence selected from

15 the group consisting of SEQ ID NOs:10-15. The invention further provides an isolated nucleic acid molecule encoding a variable light chain region of an antibody, wherein the antibody binds to human platelet glycoprotein Ib alpha and inhibits aggregation of platelets, the

20 nucleic acid molecule encoding a first amino acid sequence having at least 90% amino acid identity to a second amino acid sequence, the second amino acid sequence selected from the group consisting of SEQ ID NOs:16-21. In further embodiments, the first amino acid

25 sequence has at least 95%, 96%, 97%, 98%, or 99% amino acid identity to the recited sequence.

entention further provides an isolated variable heavy chain or a variable light chain region of an antibody, wherein the antibody binds to human platelet glycoprotein Ib

30 alpha and inhibits aggregation of platelets. The variable heavy chain is preferably encoded by a nucleotide sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 3, and SEQ ID

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NO: 4. The variable heavy chain preferably has an amino acid sequence selected from the group consisting of SEQ ID NOs:10-15. The variable light chain is preferably encoded by a nucleotide sequence selected from the group consisting of SEQ ID NOs:5-9. The variable light chain preferably has an amino acid sequence selected from the group consisting of SEQ ID NOs:16-21. Further provided is an isolated variable heavy chain region of an antibody, wherein the antibody binds to human platelet glycoprotein Ib alpha and inhibits aggregation of platelets, the variable heavy chain being encoded by a first amino acid sequence having at least 90% amino acid identity to a second amino acid sequence, the second amino acid sequence selected from the group consisting of SEQ ID NOs:10-15. Also provided is an isolated variable light chain region of an antibody, wherein the antibody binds to human platelet glycoprotein Ib alpha and inhibits aggregation of platelets, the variable light chain being encoded by a first amino acid sequence having at least 90% amino acid identity to a second amino acid sequence, the second amino acid sequence selected from the group consisting of SEQ ID NOs:16-21. In further embodiments, the first amino acid sequence has at least 95%, 96%, 97%, 98%, or 99% amino acid identity to the recited sequences.

It should be readily apparent to those skilled in the art that a met residue may need to be added to the amino terminal of the amino acid sequence of the variable heavy chain or variable light chain region or an ATG added to the 5' end of the nucleotide sequence, in order to express the variable heavy chain or variable light

chain region in a host cell. The met version of the variable heavy chain or variable light chain region is thus specifically intended to be covered by reference to particular SEQ ID NOs.

- 5 The invention further provides an antibody comprising the variable heavy chain or variable light chain region disclosed herein. Antibodies of the subject invention include monovalent, bivalent, and polyvalent antibodies, as well as fragments of these antibodies.
- 10 Fragments of the antibodies of the present invention include, but are not limited to, the Fab and the F(ab')₂ fragments.

 The antibodies of the subject invention may be provided in a detectably labeled form. Antibodies can be

15 detectably labeled through the use of radioisotopes, affinity labels (such as biotin, avidin, etc.), enzymatic labels (such as horseradish peroxidase, alkaline phosphatase, etc.), fluorescent labels (such as FITC or rhodamine, etc.), paramagnetic atoms, etc. Epitope tags

20 can also be used, such as the c-myc peptide (to which antibodies are available that recognize the small peptide or protein of interest). The 6x-histidine tag (for which there are not only antibodies available but also

25 chelating materials that have a high affinity for the histidines) is also commonly used to purify secreted proteins. Procedures for accomplishing such labeling are well known in the art, for example see Sternberger et al. 1970, Bayer et al. 1979, Engval et al. 1972, and Goding 1976.

- 30 Further provided is a composition comprising the antibody and a carrier. The composition can be used to inhibit aggregation of platelets by exposing platelets to the composition. The antibody can also be used to bind to human platelet glycoprotein Ib alpha, the method

comprising exposing human platelet glycoprotein Ib alpha to the antibody.

In the methods of the invention, tissues or cells or platelet glycoprotein Ib alpha (a cell surface protein) are contacted with or exposed to the composition or antibody of the subject invention. In the context of this invention, to "contact" tissues or cells or platelet glycoprotein Ib alpha with or to "expose" tissues or cells or platelet glycoprotein Ib alpha to a composition or antibody means to add the composition or antibody, usually in a liquid carrier, to a cell suspension or tissue sample, either in vitro or ex vivo, or to administer the composition or antibody to cells or tissues within an animal (including humans).

The formulation of therapeutic compositions and their subsequent administration is within the skill in the art. In general, for therapeutics, a patient suspected of needing such therapy is given a composition in accordance with the invention, commonly in a pharmaceutically acceptable carrier, in amounts and for periods which will vary depending upon the nature of the particular disease, its severity and the patient's overall condition. The pharmaceutical compositions of the present invention may be administered in a number of ways depending upon whether local or systemic treatment is desired and upon the area to be treated. Administration may be topical (including ophthalmic, vaginal, rectal, intranasal, transdermal), oral or parenteral. Parenteral administration includes intravenous drip or infusion, subcutaneous, intraperitoneal or intramuscular injection, pulmonary administration, e.g., by inhalation or insufflation, or intrathecal or intraventricular administration.

Formulations for topical administration may include transdermal patches, ointments, lotions, creams, gels, drops, suppositories, sprays, liquids and powders. Conventional pharmaceutical carriers, aqueous, powder or
5 oily bases, thickeners and the like may be necessary or desirable. Coated condoms, gloves and the like may also be useful.

Compositions for oral administration include powders or granules, suspensions or solutions in water or non-
10 aqueous media, capsules, sachets or tablets. Thickeners, flavoring agents, diluents, emulsifiers, dispersing aids or binders may be desirable.

Compositions for parenteral, intrathecal or intraventricular administration may include sterile
15 aqueous solutions which may also contain buffers, diluents and other suitable additives.

In addition to such pharmaceutical carriers, cationic lipids may be included in the formulation to facilitate uptake. One such composition shown to
20 facilitate uptake is LIPOFECTIN (BRL, Bethesda MD).

Dosing is dependent on severity and responsiveness of the condition to be treated, with course of treatment lasting from several days to several months or until a cure is effected or a diminution of disease state is
25 achieved. Optimal dosing schedules can be calculated from measurements of drug accumulation in the body and from assessment of the function of platelets obtained from blood specimens from the patient. Persons of ordinary skill can easily determine optimum dosages,
30 dosing methodologies and repetition rates. Optimum dosages may vary depending on the relative potency of individual compositions, and can generally be calculated based on IC_{50} 's or EC_{50} 's in in vitro and in vivo animal studies. For example, given the molecular weight of

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compound (derived from oligonucleotide sequence and/or chemical structure) and an effective dose such as an IC_{50} , for example (derived experimentally), a dose in mg/kg is routinely calculated.

5 Once a variable heavy chain or variable light chain of interest is identified, the antibody constructed using the variable heavy chain or variable light chain be used to identify peptides capable of mimicking the inhibitory activity of the antibody. One such method utilizes the
10 development of epitope libraries and biopanning of bacteriophage libraries. Briefly, attempts to define the binding sites for various monoclonal antibodies have led to the development of epitope libraries. Parmley and Smith developed a bacteriophage expression vector that
15 could display foreign epitopes on its surface (Parmley, S.F. & Smith, G.P., Gene 73:305-318 (1988)). This vector could be used to construct large collections of bacteriophage which could include virtually all possible sequences of a short (e.g. six-amino-acid) peptide. They
20 also developed biopanning, which is a method for affinity-purifying phage displaying foreign epitopes using a specific antibody (see Parmley, S.F. & Smith, G.P., Gene 73:305-318 (1988); Cwirla, S.E., et al., Proc Natl Acad Sci USA 87:6378-6382 (1990); Scott, J.K. &
25 Smith, G.P., Science 249:386-390 (1990); Christian, R.B., et al., J Mol Biol 227:711-718 (1992); Smith, G.P. & Scott, J.K., Methods in Enzymology 217:228-257 (1993)).

After the development of epitope libraries, Smith et al. then suggested that it should be possible to use the
30 bacteriophage expression vector and biopanning technique of Parmley and Smith to identify epitopes from all possible sequences of a given length. This led to the idea of identifying peptide ligands for antibodies by biopanning epitope libraries, which could then be used in

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vaccine design, epitope mapping, the identification of genes, and many other applications (Parmley, S.F. & Smith, G.P., Gene 73:305-318 (1988); Scott, J.K., Trends in Biochem Sci 17:241-245 (1992)).

5 Using epitope libraries and biopanning, researchers searching for epitope sequences found instead peptide sequences which mimicked the epitope, i.e., sequences which did not identify a continuous linear native
10 protein sequence. These mimicking peptides are called mimotopes. In this manner, mimotopes of various binding sites/proteins have been found.

 The sequences of these mimotopes, by definition, do not identify a continuous linear native sequence or
15 necessarily occur in any way in a naturally-occurring molecule, i.e. a naturally occurring protein. The sequences of the mimotopes merely form a peptide which functionally mimics a binding site on a naturally-occurring protein.

20 Many of these mimotopes are short peptides. The availability of short peptides which can be readily synthesized in large amounts and which can mimic naturally-occurring sequences (i.e. binding sites) offers great potential application.

25 Using this technique, mimotopes to an antibody that recognizes platelet glycoprotein Ib alpha can be identified. The sequences of these mimotopes represent short peptides which can then be used in various ways, for example as peptide drugs that bind to platelet
30 glycoprotein Ib alpha and inhibit aggregation of platelets. Once the sequence of the mimotope is determined, the peptide drugs can be chemically synthesized.

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The antibodies of the subject invention (or fragments thereof) can thus be used to select peptides, mimotopes, etc. that are complementary to the antibodies and that can then be used as antidotes to the antibodies themselves. For example, if a subject being treated with the antibody in order to inhibit platelet aggregation was involved in a motor vehicle accident and the risk of bleeding far exceeded the risk of thrombosis, it would be desirable to turn "off" the antibody of the subject invention. This could be done by using the peptide or mimotope to the antibody itself. The peptide or mimotope could thus be administered to the subject to displace the antibody from the platelets, preventing the antibody-induced inhibition of platelets.

15 The identified variable heavy chain and variable light chain regions of an antibody, wherein the antibody inhibits aggregation of platelets, can also be used to select additional variable heavy chain and variable light chain regions of an antibody which inhibits aggregation of platelets. Such a method involves the selection of a variable heavy chain or variable light chain region as defined above (for example, SEQ ID NOs:10-15 for heavy chains, SEQ ID NOs:16-21 for light chains), wherein each of the variable heavy chain or variable light chain regions has an amino acid sequence; altering the amino acid sequence of the selected variable heavy chain or variable light chain region; constructing an antibody having the altered amino acid sequence of the variable heavy chain or variable light chain region; and

25 determining whether the antibody inhibits aggregation of platelets, wherein the altered variable heavy chain or variable light chain region of an antibody that inhibits aggregation of platelets is thereby selected.

30

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EXAMPLE I

Full DNA sequences were isolated from the Human Synthetic VH and VL ScFv Library (the Griffin.1 Library, available from the Medical Research Council in England), and the protein sequences of multiple ScFv clones were determined. The ScFv clones were selected on the basis of their binding to platelet GPIb. Whether displayed as surface proteins on the phagemid or secreted as free ScFv by *E. coli*, several of these different ScFv clones have proven capable of inhibiting von Willebrand factor (vWF)-dependent aggregation of platelets, most likely due to their altering the binding site for vWF that is known to be contained within GPIb. Since the Griffin.1 Library was constructed from native human antibody heavy and light chain variable sequences, ScFv isolated from this library are comprised of native human protein sequences - and hence very attractive potential reagents for therapeutic purposes. The ScFv provide a new class of anti-thrombotic agents, useful for the prevention of platelet-dependent thrombi in diseased arteries, bypass grafts, dialysis access, etc. In contrast to antibodies derived from mouse or other species, the human ScFv stand a far better chance of being recognized as self, rather than as a foreign protein.

In addition to the potential anti-thrombotic uses of the isolated ScFv, these ScFv are also useful as diagnostic reagents in human medicine. Since GPIb is a highly restricted antigen in its expression throughout the body, it has turned out to be one of the best markers to identify platelets, their precursor cell (the megakaryocyte), and leukemic blast cells of megakaryocytic origin. Additionally, there is some evidence in the literature that assaying a soluble form of platelet GPIb in the plasma (that presumably results

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from proteolytic degradation of platelet surface GPIb) may be useful as a clinical marker. The new anti-GPIb ScFv are readily harvested from *E.coli* cultures, rather than from the mammalian cells required for murine
5 monoclonal antibodies, and may therefore be a more economical source of anti-GPIb markers for diagnostic uses than were previously available.

Since the human ScFv are directed against platelet glycoprotein Ib, they have been named H1b-1, H1b-2, H1b-
10 3, etc., so as to reinforce the source of the ScFv (Human) and the target of the ScFv (Ib). In the case of H1b-1, H1b-2, and H1b-3, DNA sequencing has provided the amino acid sequences of both the heavy chain and light chain variable regions contributing to the ScFv,
15 including VH exon, particular VH CDR3, JH, linker sequence, VL exon, particular VL CDR3, and JL segments. In the case of H1b-5 and H1b-6, DNA sequencing has thus far provided the amino acid sequences of the light chain variable regions contributing to the ScFv.

20 This technology provides advantages over existing technology, including:

1. For therapeutic purposes, an anti-platelet antibody of human sequence may obviate the human anti-mouse antibody reaction seen when murine antibodies are used.
- 25 2. Platelet GPIb is an important target for anti-thrombotics that is only now beginning to be appreciated.
2. The ScFv are produced by bacterial cultures, which potentially may be more economical than the mammalian cell cultures required for murine antibodies.
- 30 3. Since the ScFv are fully cloned, the opportunity to make modifications of the basic ScFv molecules is readily available.
4. Since these ScFv were selected without immunization of animals, it is possible that one or more of these ScFv

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is directed against an epitope within GPIb for which animals might fail to mount an immune response, due to a high degree of structural conservation across species lines. The Griffin.1 library was constructed in such a manner that ScFv directed against normal human antigens are also included in the repertoire.

The ScFv clones were obtained by screening the Griffin.1 Library. The key points in this screening process were that the first steps in the screening procedure utilized CHO cells expressing recombinant GPIb alpha, and then applicant took the subset of the library surviving three rounds of selection against these cells, and then applicant went into a 4th round against normal washed human platelets. Applicant then did two final rounds where applicant attempted to displace ScFv from washed platelets by flooding them with a lot of murine monoclonal antibody (C-34 or SZ-2) or mimotope peptide (AWNWRYYREYV).

The human synthetic V_H and V_L ScFv library was made by recloning the heavy and light chain variable regions from the lox library vectors (Griffiths et al. 1994) into the phagemid vector pHEN2 (see Fig. 1). This "Griffin.1" library is a ScFv phagemid library made from synthetic V-gene segments. The World Wide Web address to download the germline V gene sequences which comprise the Griffin.1 library is <http://www.mrc-cpe.cam.ac.uk/imt-doc/vbase-questions.html>. The kappa and lambda light chain variable regions were PCR amplified from the fdDOG-2loxVk and VL constructs. The PCR fragments were purified and digested with ApaI and Not I. The gel purified fragments were then ligated into the vector pHEN2. Heavy chain variable regions were PCR amplified from the pUC19-2loxVH vector. The PCR fragments were purified and digested with SfiI and XhoI. The gel

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purified fragments were then ligated into the vector Vκ-pHEN2 or VL-pHEN2.

The isolation of the H1b series of ScFv was performed as follows:

- 5 Initial three rounds of phagemid selection against transfected Chinese Hamster Ovary (CHO) cells expressing only the GPIb alpha component of the GPIb/IX/V complex on their surface
 - 10
 - 10^{12} - 10^{13} phagemid incubated 1.5 hours at RT with transfected CHO cells adherent to culture flask
 - Unbound phagemid removed by extensive washing
 - Bound phagemid eluted with triethylamine, neutralized with Tris, infected into *E. coli* suppressor strain TG1, and amplified (using
 - 15 helper phage) for use in next round
 - Monoclonal phagemid clone *H1b-3* is a representative clone from this stage of selection
- Round 4 of selection: Against washed human platelets
 - 20
 - 10^{12} phagemid incubated with a suspension of washed platelets for 1.5 hours at RT
 - Unbound phagemid removed by extensive washing of platelets
 - Bound phagemid eluted with triethylamine,
 - 25 neutralized with Tris, infected into *E. coli*, and amplified for use in next round
 - Monoclonal phagemid clone *H1b-3* is a representative clone from this stage of selection
- 30 Rounds 5 and 6 of selection: Displacement of phage bound to platelets (optional)
 - Round 5: 10^{12} Phage from round four incubated with 3×10^9 washed platelets, unbound phage

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removed by extensive washing, and platelets then divided into three aliquots

- Incubation of platelets for 90 min at RT with 25 μ g/mL anti-GPIb alpha murine mabs C-34 or SZ-2 or 200 μ g/mL C-34 mimotope peptide AWNWRYREYV
- Phagemid recovered from buffer then infected into *E. coli*, and amplified for use in next round
- Round 6: amplified phage from fifth round bound to washed platelets and then challenged with same potential displacer as used in round 5

Production of ScFv from Round 6 Phagemids

- Phagemid recovered from round 6 infected into *E. coli* non-suppressor strain HB2151
- 24-well monoclonal culture supernatants assayed in Western blots against platelet lysates and for ability to inhibit ristocetin-induced aggregation of washed human platelets
- Interesting clones scaled up for DNA sequencing and further functional studies on purified ScFv

This work employs ScFv technology in the development of a new family of antibody molecules directed against human platelet GPIb α --molecules that are themselves derived from human immunoglobulin variable sequences. As an alternative to natural IgG antibodies, synthetic monovalent antibodies with increasingly high affinities can be made from the heavy chain variable and light chain variable regions, separated by a linker region. Such synthetic variable antibodies are termed ScFv. The Griffin.1 synthetic ScFv library, composed of human germline VH and VL sequences used for these studies was

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produced by the laboratory of Dr. Greg Winter of the MRC in Cambridge, UK. As posted on this laboratory group's web page (<http://www.mrc-cpe.cam.ac.uk/-phage/>), "This library contains exactly the same synthetic human V-genes
5 as the Human Synthetic Fab (4-12) 2lox Library (Griffiths, A.D. et al., (1994). EMBO J. 13, 3245-3260) but is in a single chain Fv (scFv) format instead of an Fab format. The vector is also a phagemid rather than a phage so it is like the Human Synthetic ScFv Library or
10 "Nissim Library" (Nissim, A. et al., (1994). EMBO J. 13, 692-698) but with diversity in the light chains as well as the heavy chains." In addition to the in vitro recombination of heavy and light chains, this library has achieved an estimated total diversity of 1.2×10^9 clones
15 through in vitro randomization at the hypervariable CDR3 regions. The resulting VH and VL coding sequences were then cloned into the pHEN2 phagemid. Depending upon whether the phagemid is infected into a strain of E.coli lacking or possessing a suppressor for the amber codon,
20 one can then obtain either progeny phagemid expressing the ScFv in fusion with a major phage coat protein, or a secreted form of the ScFv. The secreted ScFv also contain a 6x-His tag which can be used in protein purification and a c-myc tag for detection with an anti-
25 c-myc antibody such as 9E10.

For the present studies, the initial round of phagemid selection was performed against transfected Chinese Hamster Ovary (CHO) cells expressing only the GPIIb component of the GPIIb/IX/V complex on their
30 surface. 10^{12} - 10^{13} phagemid were incubated for 1.5 hours at RT with transfected CHO cells adherent to the culture flask. Unbound phagemid were removed by extensive washing, and bound phagemid were eluted with triethylamine, neutralized with Tris, infected into the

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E. coli suppressor strain TG1, and then amplified (using helper phage) for use in the next round. This process was then repeated for two additional rounds.

For the 4th round of selection, we performed a
5 "crossover" step, using human platelets. We aimed by this approach to significantly enrich for phagemid recognizing epitopes present only on both the CHO cell and the platelet, thereby increasing the odds of finding ScFv with specificities for GPIb α . Monoclonal phagemid
10 clone H1b-3 is a representative clone from this stage of selection. Whereas the polyclonal collection of all 4th round phagemid did identify GPIb α in Western blots, most individual clones tested did not. Moreover, random
conversion of Round 4 phagemid clones to soluble ScFv did
15 not yield ScFv that exhibited inhibitory activity in functional assays.

We accordingly proceeded to additional rounds, designed so as to try to direct the selection of epitopes most relevant to the vWF binding function of GPIb α .
20 Towards this end, phage from round 4 were incubated with washed platelets, and unbound phage removed by extensive washing. Platelets were then further incubated with saturating concentrations of the anti-GPIb α murine mabs C-34 or SZ-2 or with C-34 mimotope peptide, which we have
25 previously shown to compete with platelets for binding to C-34.. Phage recovered in the buffer following these incubations were amplified, and then used in a 6th and final round, in which displacement of phage was again attempted with the same mab or peptide used with it in
30 the previous round.

Phagemid recovered from round 6 were directly infected into the E. coli non-suppressor strain HB2151. Secreted ScFv from overnight supernatants were assayed in Western blots against platelet lysates and were tested

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for their ability to inhibit ristocetin-induced aggregation of washed human platelets. Interesting clones were then chosen for further study.

A particularly prominent clone (H1b-1) was observed whether SZ-2, C-34, or C-34 mimotope peptide was used as displacer. Clone H1b-2 was uniquely seen when SZ-2 was used as displacer. Clone H1b-3, as stated above, was derived from an earlier round of selection. Clones H1b-5 and H1b-6 were recovered in the buffer when C-34 mimotope peptide was used as displacer.

The purified H1b ScFv, whether purified from culture supernatants or from periplasmic spaces, had the anticipated molecular weight of 29 kilodaltons. This is illustrated in Fig. 10, where both crude supernatant and purified periplasmic fraction from an E. coli culture were run on SDS-polyacrylamide gel electrophoresis (SDS-PAGE), electroblotted, and then incubated with the murine monoclonal antibody 9E10, which recognizes the c-myc epitope tag contained within the secreted ScFv. Peroxidase-conjugated secondary anti-mouse antibody was then used to detect the presence of bound 9E10.

The binding specificity of selected clones with respect to epitope targets deriving from human platelets was also established by immunoblotting. Detergent lysates of platelets obtained from human blood were prepared, and were electrophoresed by SDS-PAGE either under non-reducing conditions or following reduction with b-mercaptoethanol. GPIb α has an apparent molecular mass of 135-140 kDa when electrophoresed under reducing conditions in this system, but characteristically migrates with an apparent molecular mass in the 160-170 kDa region under non-reducing conditions, reflecting the additional mass of GPIbB with which it is covalently bonded in the non-reduced, native state. Following

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electroblotting to a membrane, the electrophoresed platelet lysates were then probed with either the well-established anti-GPIb α murine monoclonal antibody SZ-2, or with a product from one of the selected clones.

5 Detection of binding of the SZ-2 employed a peroxidase-labeled secondary anti-mouse antibody, as described above. In the earlier rounds of selection, phagemid from selected clones were directly incubated with the blots, and following washings, the residual binding of phagemid

10 was detected through the use of an anti-M13 bacteriophage antibody, since the M13 surface epitope for this antibody is preserved in the pHEN2 phagemid. This approach permitted ready distinction between clones of phagemid that mimicked the binding pattern seen with SZ-2 from

15 those that did not. Following the later rounds of selection, however, when secreted clonal ScFv became available, the ScFv were used instead of actual phagemid in the immunoblotting. Thus, ScFv secreted by clones H1b-1, H1b-2, H1b-5, and H1b-6, when used as the primary

20 antibody in a Western blot against human platelet lysates, all showed a pattern closely mimicking that of murine monoclonal antibody SZ-2. An example of this is shown in Fig. 11, for H1b-1 and H1b-2 ScFv. Following the initial incubation with and subsequent washings of

25 the membranes with the indicated ScFv, secondary antibody 9E10, and in turn peroxidase-conjugated anti-mouse antibody were incubated with the membrane, and staining developed with peroxidase substrate. As can be seen in this example, the products of the selected clones were

30 able to recognize bands having the migration characteristics of GPIb α , both under non-reducing and reducing conditions.

The ability of products from the selected clones to inhibit platelet function was tested by platelet

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aggregation. Since a major function of GPIIb/IIIa is its role as receptor for the adhesive ligand, von Willebrand factor (vWF), vWF-dependent platelet aggregation was of particular interest. In vitro, aggregation involving the binding of vWF to platelet GPIIb is conventionally assessed using either ristocetin or botrocetin as mediators. ScFv obtained from clones H1bB-1, H1bB-2, H1bB-5, and H1bB-6 were found to inhibit vWF-dependent platelet aggregation induced by at least one of these mediators. In the case of H1bB-3, the phagemid itself showed inhibitory activity in an aggregation assay.

A representative example of inhibition at the phagemid level is shown in Fig. 12. Human platelets that have been formalin-fixed were suspended in buffer containing 5 $\mu\text{g/mL}$ purified vWF at a final platelet concentration of 150,000/ μL , added to a cuvette with a stir bar, and stirred at 1200 rpm, 37 °C, in a Chronolog Aggregometer. Ristocetin was then added at varying final concentrations, and resulting change in light transmittance used as an indicator of platelet aggregation. As can be seen in the figure, pre-incubation for 1.5 hour of the platelets (at 150,000/ μL) with 1×10^{12} H1bB-3 phagemid totally inhibited platelet aggregation at ristocetin concentrations at or below 0.35 mg/mL in this system, and even at a ristocetin concentration as high as 1.0 mg/mL continued to exert strong inhibition. In contrast, in the presence of an equal concentration of phagemid not expressing activity against platelet GPIIb/IIIa (Control Phagemid), a full aggregatory response was reached by 0.5 mg/mL ristocetin, with moderate aggregation responses observed in the range of 0.3-0.35 mg/mL ristocetin. (Note that the formalin-fixed platelets characteristically are aggregated in the

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presence of lower concentrations of ristocetin than is usually required with non-fixed platelets.)

A representative example of inhibition at the ScFv level is shown in Fig. 13. Fixed human platelets were again used under similar conditions to those previously described. In this experiment formalin-fixed human platelets at a concentration of 150,000/ μ L were incubated for 1.5 hour with HIB ScFv at the indicated final concentration. Purified vWF was then added to a final concentration of 5 μ g/mL, the sample put in the aggregometer in the manner described above, and botrocetin added at a final concentration of 0.6 μ g/mL to initiate aggregation. In this example, the rate of light transmittance change in the aggregometer is used as an index of aggregation. Quite strong (>75%) inhibition of the aggregation response is observed when the platelets have been preincubated with 12 μ g/mL HIB-1. Half-maximal inhibition is characteristically observed in the range of 5-10 μ g/mL of HIB-1. Similar results are obtained when ristocetin is used as the agonist. HIB-2 ScFv similarly inhibit the aggregation of human platelets modulated by either ristocetin or botrocetin. HIB-2 ScFv also show half-maximal inhibition of such vWF-dependent platelet aggregation in the range of 5-10 μ g/mL ScFv, with the maximal degree of such inhibition comparable to or even exceeding that seen with HIB-1. While HIB-5 and HIB-6 also exert inhibition upon vWF-dependent platelet aggregation, the maximal degree of this inhibition has been observed to be weaker than that achieved with HIB-1 or HIB-2, reaching in the range of a 20-30% inhibition of the uninhibited aggregatory response.

A further representative example, in this instance demonstrating the ability of ScFv purified from the HIB clones to exert inhibitory activity upon unfixed human

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platelets, is shown in Fig. 14. Here the inhibitory effects of a 1 hour incubation of platelets (150,000 platelets/ μ L) with either HIB-2 ScFv or with the intact (i.e., full IgG) murine monoclonal antibody SZ-2 are directly compared. In this example, platelet-rich plasma (P.P.) was prepared by centrifugation of citrated, freshly drawn human blood, and the P.P. then studied in the platelet aggregometer under similar conditions as described above. It can be seen that HIB-2 by a concentration of 10 μ g/mL was able to produce a degree of aggregation quite comparable to that seen with SZ-2 at the same final concentration.

This work thus demonstrates the discovery of a group of ScFv selected upon the basis of the selection strategy described above, that have been found to inhibit vWF-dependent platelet aggregation induced by botrocetin or ristocetin, and that in fact specifically recognize epitopes within human platelet GPIIb/IIIa that survive SDS denaturation as well as reduction with mercaptoethanol.

Although preferred embodiments have been depicted and described in detail herein, it will be apparent to those skilled in the relevant art that various modifications, additions, substitutions and the like can be made without departing from the spirit of the invention and these are therefore considered to be within the scope of the invention as defined in the claims which follow.

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What Is Claimed Is:

1 1. A method of selecting a clone that binds to
2 human platelet glycoprotein Ib alpha using a human
3 variable heavy chain and variable light chain
4 immunoglobulin library, the method comprising:
5 incubating a human variable heavy chain and variable
6 light chain immunoglobulin library with cells expressing
7 human platelet glycoprotein Ib, and selecting clones of
8 the library which bind to the cells; and
9 incubating the selected clones of the library with
10 washed human platelets, and selecting resulting clones
11 which bind to the washed human platelets, wherein the
12 resulting clones bind to human platelet glycoprotein Ib
13 alpha.

1 2. The method of claim 1 wherein the cells are
2 Chinese Hamster Ovary cells.

1 3. The method of claim 1 further comprising
2 incubating the selected resulting clones with further
3 platelets and adding an anti-glycoprotein Ib alpha
4 molecule that may displace clones already bound to the
5 further platelets, and selecting the then-resulting
6 clones that are not bound to the further platelets, the
7 then-resulting clones being capable of binding to human
8 platelet glycoprotein Ib alpha.

1 4. The method of claim 3 wherein the anti-
2 glycoprotein Ib molecule is a murine monoclonal antibody.

1 5. The method of claim 3 wherein the anti-
2 glycoprotein Ib molecule is a peptide.

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1 6. The method of claim 5 wherein the peptide has
2 an amino acid sequence as shown in SEQ ID NO:1.

1 7. An isolated nucleic acid molecule encoding a
2 variable heavy chain or a variable light chain region of
3 an antibody, or a fragment thereof, wherein the antibody
4 binds to human platelet glycoprotein Ib alpha and
5 inhibits aggregation of platelets.

1 8. The nucleic acid molecule of claim 7 wherein
2 the nucleic acid molecule encodes a variable heavy chain
3 region and has a nucleotide sequence selected from the
4 group consisting of SEQ ID NO:2, SEQ ID NO:3, and SEQ ID
5 NO:4.

1 9. The nucleic acid molecule of claim 7 wherein
2 the nucleic acid molecule encodes a variable light chain
3 region and has a nucleotide sequence selected from the
4 group consisting of SEQ ID NO:5, SEQ ID NO:6, SEQ ID
5 NO:7, SEQ ID NO:8 and SEQ ID NO:9.

1 10. The nucleic acid molecule of claim 7 wherein
2 the nucleic acid molecule encodes a variable heavy chain
3 region having an amino acid sequence selected from the
4 group consisting of SEQ ID NO:10, SEQ ID NO:11, SEQ ID
5 NO:12, SEQ ID NO:13, SEQ ID NO:14 and SEQ ID NO:15.

1 11. The nucleic acid molecule of claim 7 wherein
2 the nucleic acid molecule encodes a variable light chain
3 region having an amino acid sequence selected from the
4 group consisting of SEQ ID NO:16, SEQ ID NO:17, SEQ ID
5 NO:18, SEQ ID NO:19, SEQ ID NO:20, and SEQ ID NO:21.

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1 12. The nucleic acid molecule of claim 7 wherein
2 the nucleic acid molecule encodes a fragment of a
3 variable heavy chain region.

1 13. The nucleic acid molecule of claim 12 wherein
2 the fragment is a VH3 fragment having an amino acid
3 sequence selected from the group consisting of SEQ ID
4 NO:27, SEQ ID NO:28, SEQ ID NO:31, SEQ ID NO:32, SEQ ID
5 NO:35 and SEQ ID NO:36.

1 14. The nucleic acid molecule of claim 12 wherein
2 the fragment is a CDR1 fragment having an amino acid
3 sequence selected from the group consisting of SEQ ID
4 NO:29, SEQ ID NO:33 and SEQ ID NO:37.

1 15. The nucleic acid molecule of claim 12 wherein
2 the fragment is a CDR2 fragment having an amino acid
3 sequence selected from the group consisting of SEQ ID
4 NO:30, SEQ ID NO:34 and SEQ ID NO:38.

1 16. The nucleic acid molecule of claim 12 wherein
2 the fragment is a CDR3 fragment having an amino acid
3 sequence selected from the group consisting of SEQ ID
4 NO:39, SEQ ID NO:40 and SEQ ID NO:41.

1 17. The nucleic acid molecule of claim 7 wherein
2 the nucleic acid molecule encodes a fragment of a
3 variable light chain region.

1 18. The nucleic acid molecule of claim 17 wherein
2 the fragment has an amino acid sequence selected from the
3 group consisting of SEQ ID NO:42, SEQ ID NO:46, SEQ ID
4 NO:47, SEQ ID NO:51, SEQ ID NO:59, SEQ ID NO:60 and SEQ
5 ID NO:61.

1 19. The nucleic acid molecule of claim 17 wherein
2 the fragment is a CDR1 fragment having an amino acid
3 sequence selected from the group consisting of SEQ ID
4 NO:43, SEQ ID NO:48, SEQ ID NO:52 and SEQ ID NO:55.

1 20. The nucleic acid molecule of claim 17 wherein
2 the fragment is a CDR2 fragment having an amino acid
3 sequence selected from the group consisting of SEQ ID
4 NO:44, SEQ ID NO:49, SEQ ID NO:53 and SEQ ID NO:56.

1 21. The nucleic acid molecule of claim 17 wherein
2 the fragment is a CDR3 fragment having an amino acid
3 sequence selected from the group consisting of SEQ ID
4 NO:45, SEQ ID NO:50, SEQ ID NO:54, SEQ ID NO:57 and SEQ
5 ID NO:58.

1 22. A recombinant cell comprising the nucleic acid
2 molecule of claim 7.

1 23. The recombinant cell of claim 22 wherein the
2 cell is a bacterial cell.

1 24. An expression vector comprising the nucleic
2 acid molecule of claim 7.

1 25. The expression vector of claim 24 wherein the
2 vector is a phagemid.

1 26. A recombinant cell comprising the expression
2 vector of claim 24.

1 27. A method of producing a variable heavy chain or
2 variable light chain region of an antibody, or a fragment

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3 thereof, to human platelet glycoprotein Ib alpha that
4 inhibits aggregation of platelets, the method comprising:
5 introducing the nucleic acid molecule of claim 7
6 into a host cell; and
7 allowing the host cell to express the nucleic acid
8 molecule resulting in the production of a variable heavy
9 chain or variable light chain region of an antibody in
10 the cell.

1 28. An isolated nucleic acid molecule encoding a
2 variable heavy chain region of an antibody that binds to
3 human platelet glycoprotein Ib alpha and inhibits
4 aggregation of platelets, the nucleic acid molecule
5 encoding a first amino acid sequence having at least 90%
6 amino acid identity to a second amino acid sequence, the
7 second amino acid sequence selected from the group
8 consisting of SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12,
9 SEQ ID NO:13, SEQ ID NO:14 and SEQ ID NO:15.

1 29. An isolated nucleic acid molecule encoding a
2 variable light chain region of an antibody that binds to
3 human platelet glycoprotein Ib alpha and inhibits
4 aggregation of platelets, the nucleic acid molecule
5 encoding a first amino acid sequence having at least 90%
6 amino acid identity to a second amino acid sequence, the
7 second amino acid sequence selected from the group
8 consisting of SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18,
9 SEQ ID NO:19, SEQ ID NO:20, and SEQ ID NO:21.

1 30. An isolated variable heavy chain or variable
2 light chain region of an antibody, or a fragment thereof,
3 wherein the antibody binds to human platelet glycoprotein
4 Ib alpha and inhibits aggregation of platelets.

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1 31. The variable heavy chain region of an antibody
2 of claim 30, wherein the variable heavy chain region has
3 an amino acid sequence selected from the group consisting
4 of SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID
5 NO:13, SEQ ID NO:14 and SEQ ID NO:15.

1 32. The variable light chain region of an antibody
2 of claim 30, wherein the variable light chain region has
3 an amino acid sequence selected from the group consisting
4 of SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID
5 NO:19, SEQ ID NO:20, and SEQ ID NO:21.

1 33. A fragment of the variable heavy chain region
2 of an antibody of claim 30.

1 34. The fragment of claim 33 wherein the fragment
2 is a VH3 fragment having an amino acid sequence selected
3 from the group consisting of SEQ ID NO:27, SEQ ID NO:28,
4 SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:35 and SEQ ID
5 NO:36.

1 35. The fragment of claim 33 wherein the fragment
2 is a CDR1 fragment having an amino acid sequence selected
3 from the group consisting of SEQ ID NO:29, SEQ ID NO:33
4 and SEQ ID NO:37.

1 36. The fragment of claim 33 wherein the fragment
2 is a CDR2 fragment having an amino acid sequence selected
3 from the group consisting of SEQ ID NO:30, SEQ ID NO:34
4 and SEQ ID NO:38.

1 37. The fragment of claim 33 wherein the fragment
2 is a CDR3 fragment having an amino acid sequence selected

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3 from the group consisting of SEQ ID NO:39, SEQ ID NO:40
4 and SEQ ID NO:41.

1 38. A fragment of the variable light chain region
2 of an antibody of claim 30.

1 39. The fragment of claim 38 wherein the fragment
2 has an amino acid sequence selected from the group
3 consisting of SEQ ID NO:42, SEQ ID NO:46, SEQ ID NO:47,
4 SEQ ID NO:51, SEQ ID NO:59, SEQ ID NO:60 and SEQ ID
5 NO:61.

1 40. The fragment of claim 38 wherein the fragment
2 is a CDR1 fragment having an amino acid sequence selected
3 from the group consisting of SEQ ID NO:43, SEQ ID NO:48,
4 SEQ ID NO:52 and SEQ ID NO:55.

1 41. The fragment of claim 38 wherein the fragment
2 is a CDR2 fragment having an amino acid sequence selected
3 from the group consisting of SEQ ID NO:44, SEQ ID NO:49,
4 SEQ ID NO:53 and SEQ ID NO:56.

1 42. The fragment of claim 38 wherein the fragment
2 is a CDR3 fragment having an amino acid sequence selected
3 from the group consisting of SEQ ID NO:45, SEQ ID NO:50,
4 SEQ ID NO:54, SEQ ID NO:57 and SEQ ID NO:58.

1 43. An isolated variable heavy chain region of an
2 antibody, wherein the antibody binds to human platelet
3 glycoprotein Ib alpha and inhibits aggregation of
4 platelets, the isolated variable heavy chain region
5 having a first amino acid sequence having at least 90%
6 amino acid identity to a second amino acid sequence, the
7 second amino acid sequence selected from the group

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8 consisting of SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12,
9 SEQ ID NO:13, SEQ ID NO:14 and SEQ ID NO:15.

1 44. An isolated variable light chain region of an
2 antibody, wherein the antibody binds to human platelet
3 glycoprotein Ib alpha and inhibits aggregation of
4 platelets, the isolated variable light chain region
5 having a first amino acid sequence having at least 90%
6 amino acid identity to a second amino acid sequence, the
7 second amino acid sequence selected from the group
8 consisting of SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18,
9 SEQ ID NO:19, SEQ ID NO:20 and SEQ ID NO:21.

1 45. An antibody comprising the variable heavy chain
2 or variable light chain region of claim 30, 43 or 44.

1 46. The antibody of claim 45 wherein the antibody
2 is monovalent.

1 47. The antibody of claim 45 wherein the antibody
2 is bivalent.

1 48. The antibody of claim 45 wherein the antibody
2 is polyvalent.

1 49. A composition comprising the antibody of claim
2 45 and a carrier.

1 50. A method of inhibiting aggregation of
2 platelets, the method comprising exposing platelets to
3 the composition of claim 49.

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1 51. A method of binding human platelet glycoprotein
2 Ib alpha, the method comprising exposing human platelet
3 glycoprotein Ib alpha to the antibody of claim 45.

1 52. A method of selecting a variable heavy chain or
2 variable light chain region of an antibody, wherein the
3 antibody inhibits aggregation of platelets, the method
4 comprising:
5 selecting a variable heavy chain or variable light
6 chain region of claim 30, 43 or 44, wherein each of the
7 variable heavy chain or variable light chain regions has
8 an amino acid sequence;
9 altering the amino acid sequence of the selected
10 variable heavy chain or variable light chain region; and
11 determining whether the altered variable heavy chain
12 or variable light chain region inhibits aggregation of
13 platelets, wherein the altered variable heavy chain or
14 variable light chain region that inhibits aggregation of
15 platelets is thereby selected.

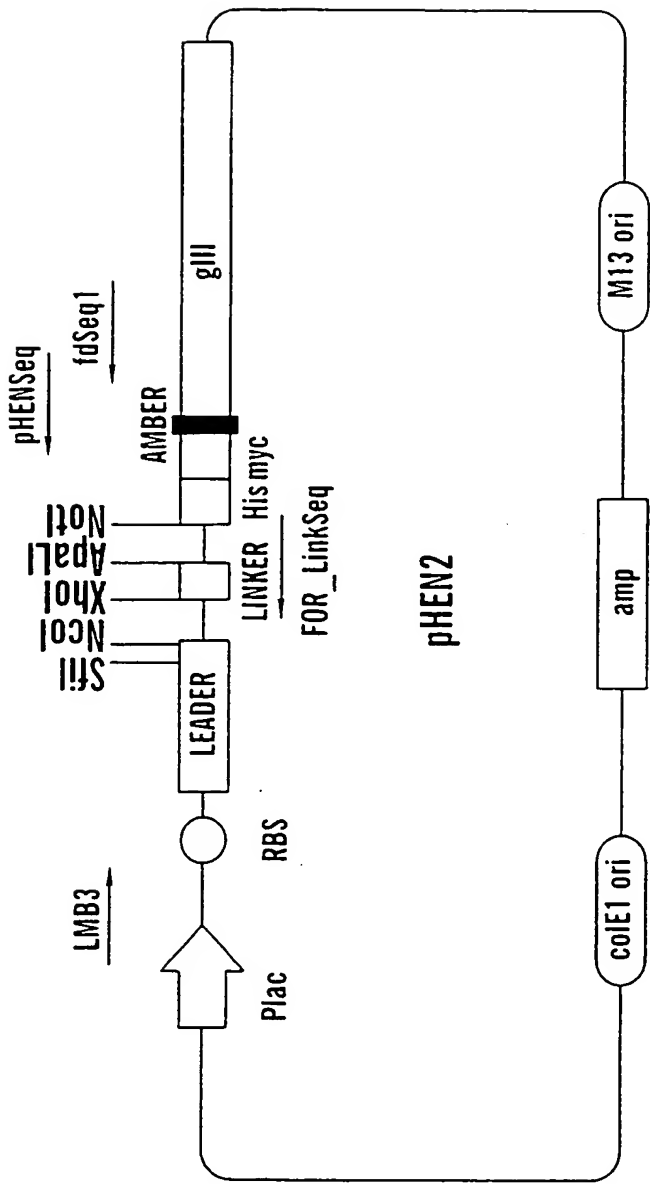
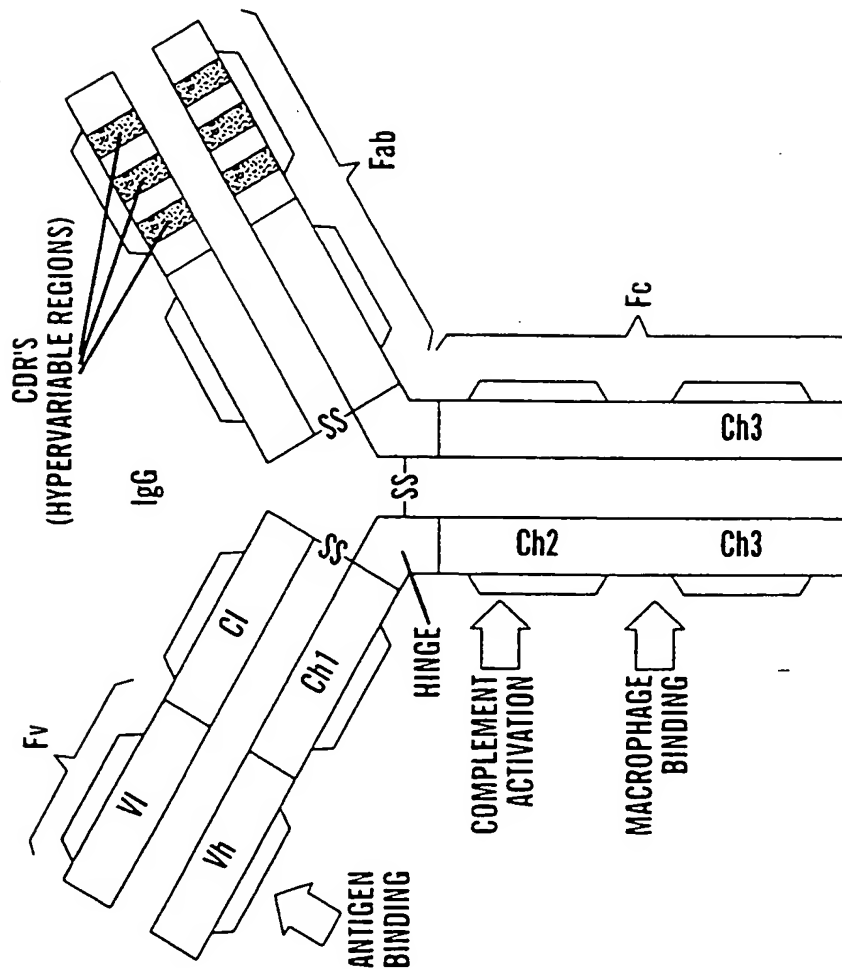
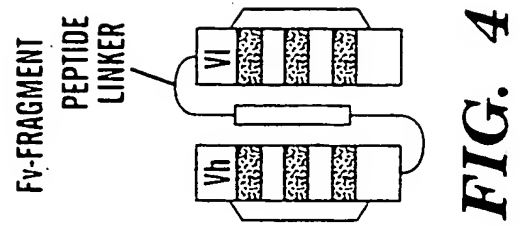
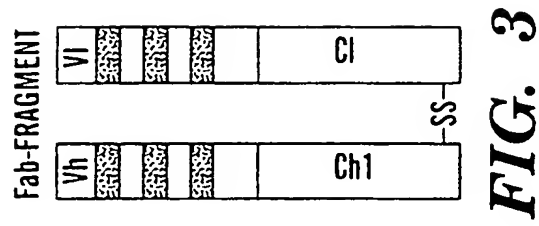


FIG. 1

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III Exon - Amino acid sequence alignment

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	FR1			FR2			FR3		
	1	2	3	4	5	6	7	8	9
III-112	123456789012345678901234567890	11112345	67890123456789	012abc3456789012345	67890123456789012abc345678901234				

VIH3 1 3 3-20 EVQIVESGGGVVRPGSLRLSCAASGFTFD 10--YGMH WVRQAPGKGLEWVS GINW--NGSGCYADSVKG RFTISRDNAKNSLYLQMNSLRDAEDTAVYYCAR} SEQ 29 (VIV)
 NOTE: For Hib 1 "VIV" replaces the "LYH" that is present at positions 89-91 within VIH3 1-3 3-20
 SEQ 29
 SEQ 30
 SEQ 28 (LYH)

6Z DAS
SEQ 30

VH CDR3: LKMPHA SEQ 39

2JH - Amino acid sequence alignment

113

{CDR}

100 110

SSAVALTGOCN--

2L Exon Amino acid sequence alignment

FRI

[illegible]

SEQ 43

SEQ 44

C_L - Amino acid sequence alignment

CUR }

100

JL3 ..VFGGGT*KL.TVL

Full Sequence of Hb-1: (Underlined linker sequence is from vector, not actual immunoglobulin chains.)

EVQLVESGGGVVPGGSLRLSCAASGFTIDYCKSWVRQAPKGLEWVSGINWNGSGTGAHPSVKRFTTISRDAKNSLYLQMNSI-RAEDTAVYCYCARLKMPHWGGQTIGGGGGSGGGSGGSAIRSEITQDPAV
SVVALGQTVIRITCGDLSRYSYASWYQKPGQAPVLIYIKGNRRPSGIPDRFSGSSSGNTASITITCAQAEADYYCNSRDSSGHNHFVGGGTKITLVIG

SEQ 22

FIG. 5

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H1b-5

VK Exon - Amino acid sequence alignment

		L1		L2		L3	
		FR1	CDR1	FR2	CDR2	FR3	CDR3
L1-L2-L3	Locus	1 2 3	4	5	6 7 8	9	
		1234567890123	4567890123456789	0123456	7890123456789012345678	9012345ab	
VKII 4-1-(1) A3		DVVMTQSPSLPVTGPGEPAISCR	RSSQSLHLS-NGYNYLD	WYLQKPGQSPQLLIY	LGSNRAS	GVDPDRFSGSGSGTDFTLKISRVEAEDVGVYYC	MOALQTPPF
			SEQ 55		SEQ 56		SEQ 57
							SEQ 58(-PF) SEQ 59 (V)(PF) SEQ 60 (I)(-PF)

NOTE: For H1b-5 "V" replaces the "I" that is present at position 2, and an additional "PF" follows position 95 within VKII 4-1(1) A3.

VK - Amino acid sequence alignment

L3
CDR3
100
TFGQGTKLEIK

Sequenced region of H1b-5: (Underlined linker sequence is from vector, not actual immunoglobulin chains.)

SSGGGGGGGGSSAL DVVMTQSPSLPVTGPGEPAISCRSSQSLHLSNGYNYLDWYLQKPGQSPQLLIYLGSNRASGVDPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQALQTPPFTFGQGTKLEIKR } SEQ 25

FIG. 8

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H1b-6

There is some homology in the light chain with VK Exon VKII 3-1-(1) O11. However, while the general structure of the VK exon appears appropriate, the number of deviations of specific amino acids from VKII 3-1-(1) O11 are so numerous, that H1b-6 would appear to have a VK unique enough to warrant receiving its own numerical assignment.

VK Exon - Amino acid sequence: EIVMTQTPLSLISITPGEQASMSCRSSQSLHSDGYTYLYWFLQKARPVSTLLICEVSNRFGVPDRFSGSGGTDFTLKISRVEAEDVGYYVYCMQDAQDP } SEQ 61

- Amino acid sequence alignment

L3
-
CDR3
-
100
|
2 -TFGQGTKLEIK

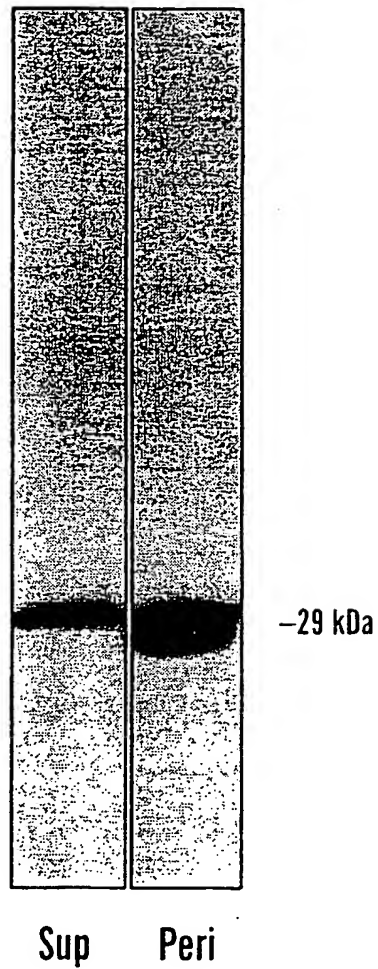
sequenced region of H1b-6: (Underlined linker sequence is from vector, not actual immunoglobulin chains.)

3GGGSGGGSGGSAIEIVMTQTPLSLISITPGEQASMSCRSSQSLHSDGYTYLYWFLQKARPVSTLLICEVSNRFGVPDRFSGSGGTDFTLKISRVEAEDVGYYVYCMQDAQDP } SEQ 26

FIG. 9

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Hib-1 Human Anti-GPIb α
DIRECT WESTERN BLOT WITH 9E10 (ANTI-c-myc)

**FIG. 10**

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WESTERN BLOT AGAINST HUMAN PLATELET LYSATES

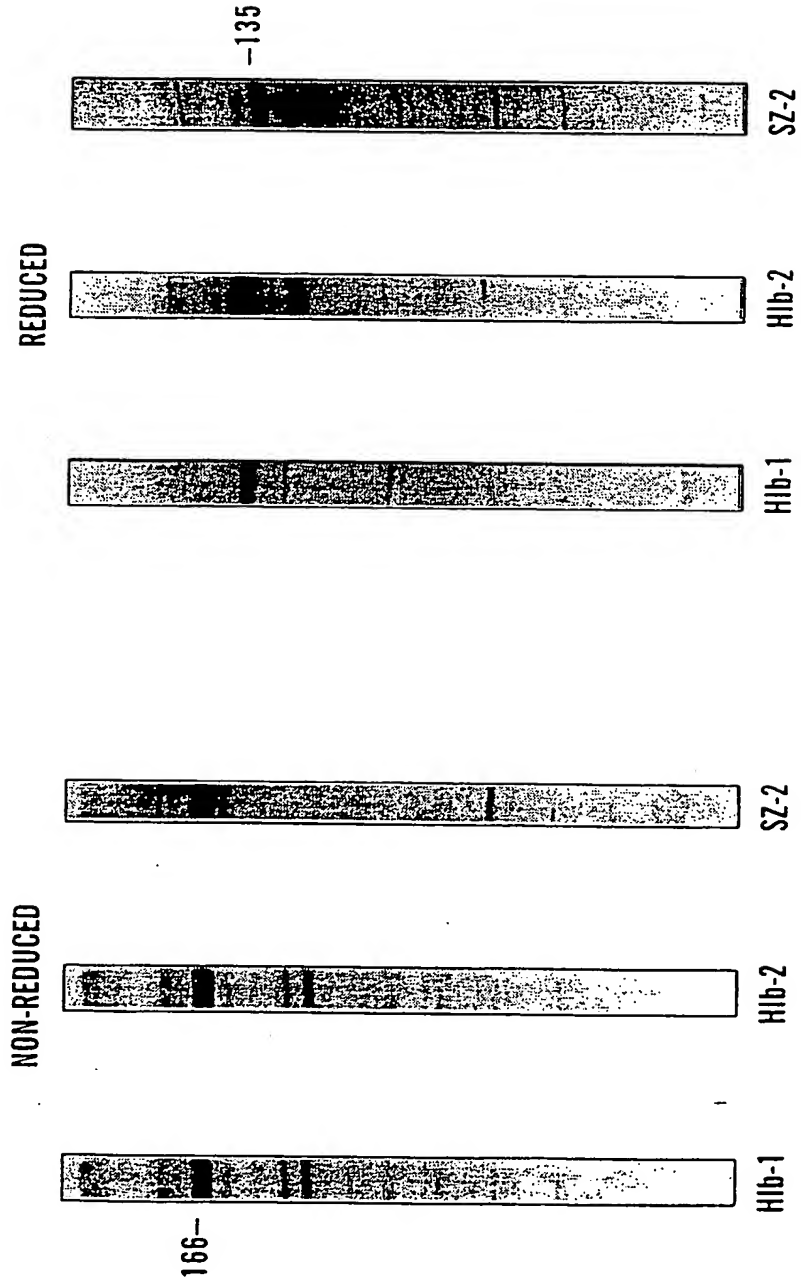


FIG. 11

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◇ — PBS
 ■ — CONTROL PHAGEMID
 × — Hib-3 PHAGEMID

INHIBITION OF RISTOCETIN-INDUCED PLATELET AGGREGATION
BY CLONAL PHAGEMID EXPRESSING HUMAN VH AND VL ScFv

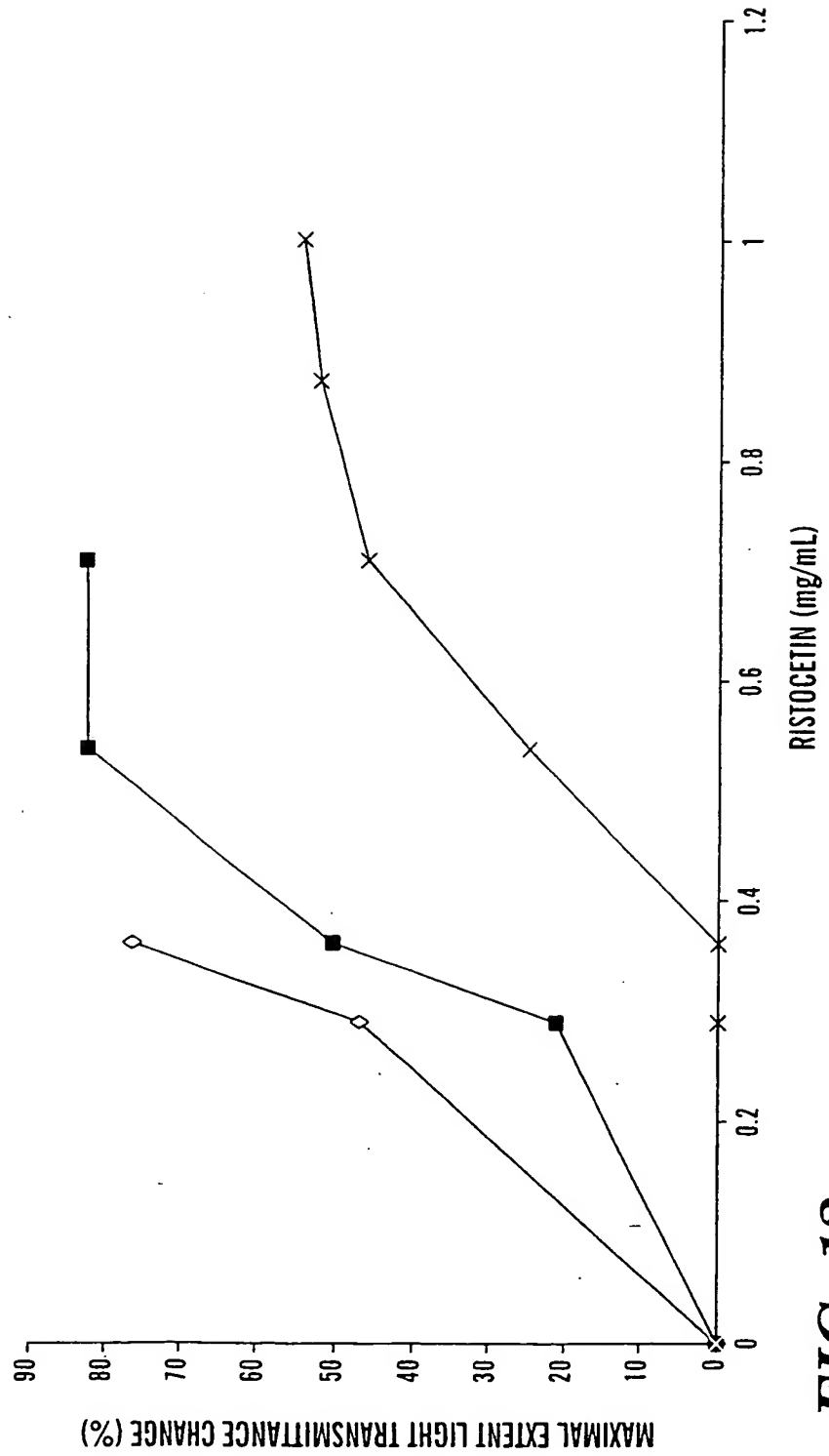


FIG. 12

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INHIBITION OF BOTROCETIN-INDUCED AGGREGATION OF FORMALIN-FIXED HUMAN PLATELETS

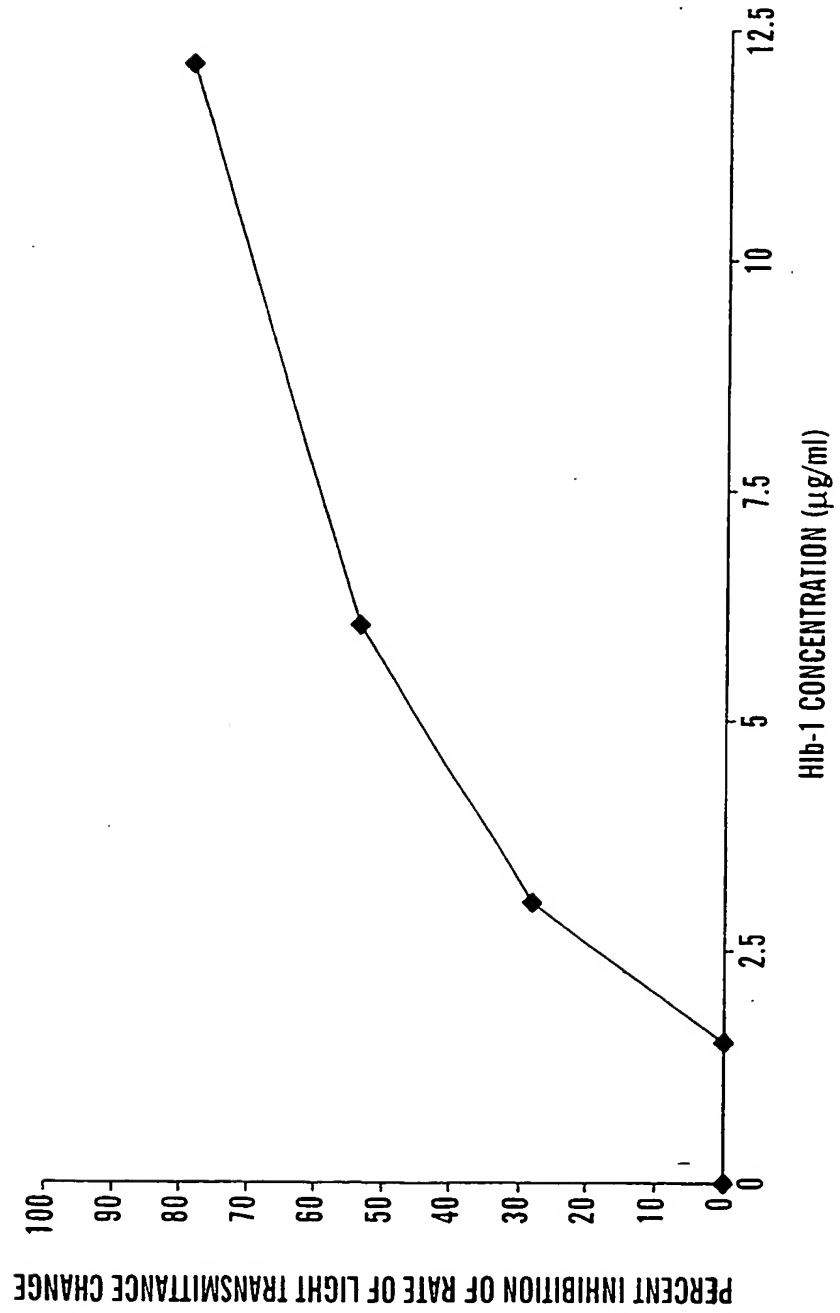


FIG. 13

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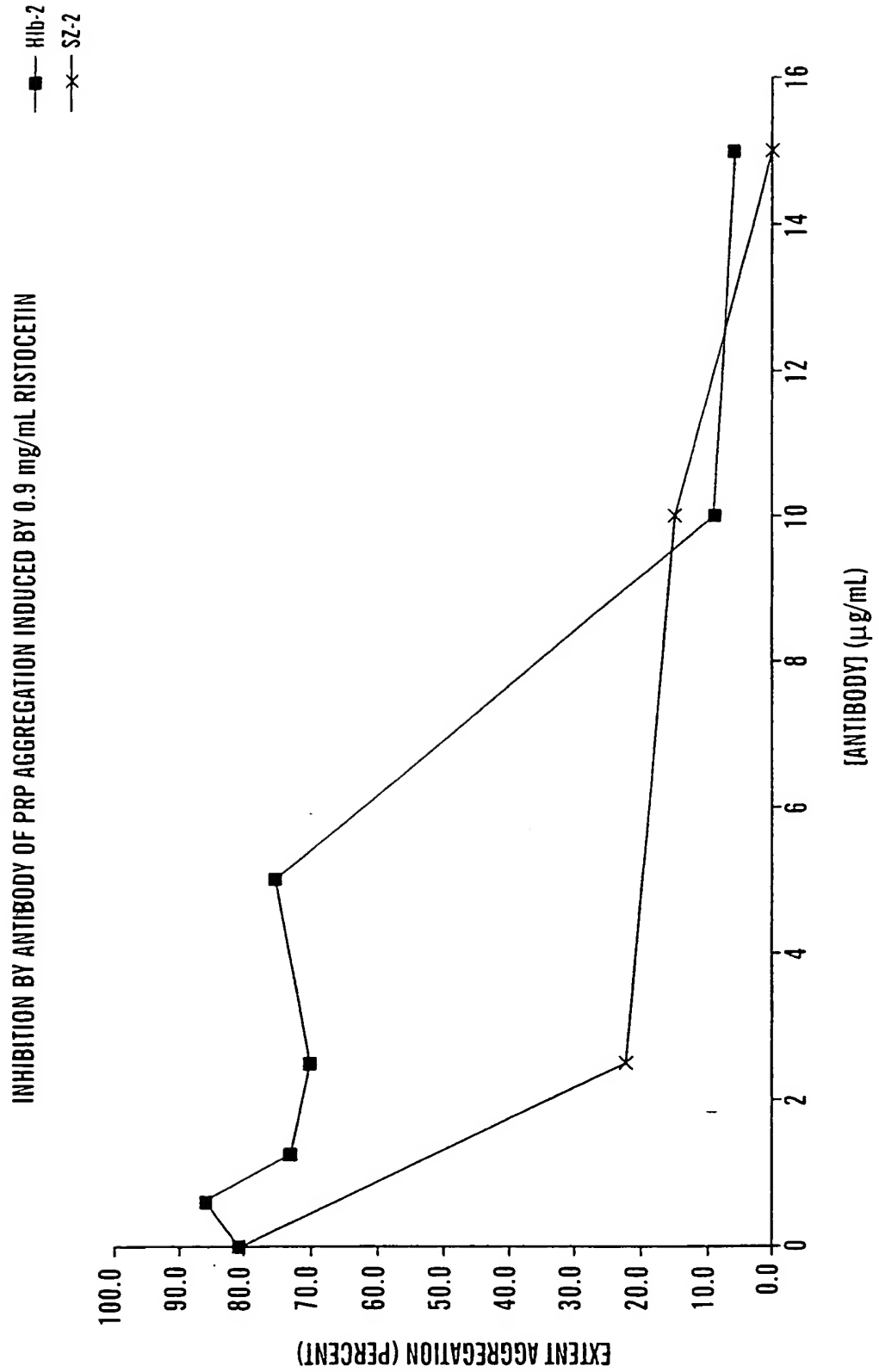


FIG. 14

SEQUENCE LISTING

<110> Miller, Jonathan L

<120> Variable Heavy Chain and Variable Light Chain Regions
of Antibodies to Human Platelet Glycoprotein Ib Alpha

<130> 027.00030

<140> PCT/US99/25495

<141> 1999-10-29

<150> US 60/106,275

<151> 1998-10-30

<160> 61

<170> PatentIn Ver. 2.1

<210> 1

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Mimotope
peptide directed to human monoclonal antibody
C-34

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1

5

10

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<211> 339

<212> DNA

<213> Homo sapiens

<400> 2

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ccagggaagg ggctggagtg ggtctccggt attaattgga atggctggtag cacagggtat 180
gcagactctg tgaagggccg attcaccatc tccagagaca acgccaagaa ctccctgtat 240
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atgcctcatg cgtggggcca aggtaccctg gtcaccgtc 339

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 <212> DNA
 <213> Homo sapiens

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 tgggtccgcc aggctccagg gaaggggctg gagtgggttg gccgtattaa aagcaaaact 120
 gatggctgga caacagacta cgtgcaccc gtgaaagcca gattcaccat ctcaagagat 180
 gattcaaaaa acacgctgta tctgcaaagt aacagcctga aaaccgagga cacggccctg 240
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<210> 4
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 <212> DNA
 <213> Homo sapiens

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 ccagggaagg ggctggagtg ggtctcagct attagtggta gtggtggtag cacatactac 180
 gcagactccg tgaaggggccg gttcaccatc tccagagaca attccaagaa cacgctgtat 240
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 ttgcttatgc tttggggcca aggtaccctg gtcacc 336

<210> 5
 <211> 324
 <212> DNA
 <213> Homo sapiens

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 caggcccttg tacttgctcat ctatggtaaa aacaaccggc cctcagggat ccagaccga 180
 tctcttggct ccagctcagg aaacacagct tccttgacca tcaactggggc tcaggcggaa 240
 gatgaggctg actattactg taactcccgg gacagcagtg gtaaccacgt attcggccga 300
 gggaccaagc tgaccgtcct aggt 324

<210> 6
 <211> 330
 <212> DNA
 <213> Homo sapiens

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gaccgattct ctggtccaa gtctggcacc tcagcctccc tggccatcag tgggtccgg 240
tccgaggatg aggtcgatta ttactgtgca gcatgggatg acagcctgtt gactgtattc 300
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<210> 7

<211> 324

<212> DNA

<213> Homo sapiens

<400> 7

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caggccctcg tacttgtcat ctatggtaaa aacaaccggc cctcagggat ccagaccga 180
tcttctggct ccagctcagg aaacacagct tccttgacca tcactggggc tcaggcgga 240
gatgaggctg actattactg taactcccg gacagcagtg gtaaccatgt attcggcgga 300
gggaccaagc tgaccgtcct aggt 324

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<210> 8

<211> 342

<212> DNA

<213> Homo sapiens

<400> 8

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tacctgcaga agccaggga gtctccacag ctctgatct atttgggttc taatcgggccc 180
tccgggggtcc ctgacaggtt cagtggcagt ggatcaggca cagattttac actgaaaatc 240
agcagagtgg aggtcgagga tgttgggtt tattactgca tgcaagcttc acaaacctcc 300
ctttttacgt tggccaagg gaccaagctg gaaatcaaac gt 342

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<210> 9

<211> 336

<212> DNA

<213> Homo sapiens

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tttctgcaga aagccaggcc agtctccacg ctctgatct gtgaagtctc caaccggctc 180
tcaggagtgc cagataggtt cagtggcagc gggtcaggga cagatttcac actgaaaatc 240
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acgttcggcc aagggaacca gctggaaatc aaacgt 336

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 <213> Homo sapiens

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 1 5 10 15
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 20 25 30
 Gly Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Ser Gly Ile Asn Trp Asn Gly Gly Ser Thr Gly Tyr Ala Asp Ser Val
 50 55 60
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
 65 70 75 80
 Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95
 Ala Arg Leu Lys Met Pro His Ala Trp Gly Gln Gly Thr Leu Val Thr
 100 105 110
 Val

<210> 11
 <211> 113
 <212> PRT
 <213> Homo sapiens

<400> 11
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 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asp Asp Tyr
 20 25 30
 Gly Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Ser Gly Ile Asn Trp Asn Gly Gly Ser Thr Gly Tyr Ala Asp Ser Val

[illegible]

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<213> Homo sapiens
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Ala Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp
      20             25             30
Val Gly Arg Ile Lys Ser Lys Thr Asp Gly Gly Thr Thr Asp Tyr Ala
      35             40             45
Ala Pro Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn
      50             55             60
Thr Leu Tyr Leu Gln Met Asn Ser Leu Lys Thr Glu Asp Thr Ala Val
      65             70             75             80
Tyr Tyr Cys Ala Arg Asn Pro Lys Leu Val Lys Trp Gly Gln Gly Thr
      85             90             95
Leu Val Thr Val
      100

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<210> 13
<211> 100
<212> PRT
<213> Homo sapiens
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<400> 13

Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asn
 1 5 10 15

Ala Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp
 20 25 30

Val Gly Arg Ile Lys Ser Lys Thr Asp Gly Gly Thr Thr Asp Tyr Ala
 35 40 45

Ala Pro Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn
 50 55 60

Thr Leu Tyr Leu Gln Met Asn Ser Leu Lys Thr Glu Asp Thr Ala Val
 65 70 75 80

Tyr Tyr Cys Thr Thr Asn Pro Lys Leu Val Lys Trp Gly Gln Gly Thr
 85 90 95

Leu Val Thr Val
 100

<210> 14

<211> 112

<212> PRT

<213> Homo sapiens

<400> 14

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30

Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45

Ser Ala Ile Ser Gly Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
 50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Ala Trp Lys Ser Leu Leu Met Leu Trp Gly Gln Gly Thr Leu Val Thr

100

105

110

<210> 15
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 <212> PRT
 <213> Homo sapiens

<400> 15
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 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30
 Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Ser Ala Ile Ser Gly Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
 50 55 60
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80
 Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95
 Ala Arg Lys Ser Leu Leu Met Leu Trp Gly Gln Gly Thr Leu Val Thr
 100 105 110

<210> 16
 <211> 108
 <212> PRT
 <213> Homo sapiens

<400> 16
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 Thr Val Arg Ile Thr Cys Gln Gly Asp Ser Leu Arg Ser Tyr Tyr Ala
 20 25 30

Ser Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Val Leu Val Ile Tyr
35 40 45

Gly Lys Asn Asn Arg Pro Ser Gly Ile Pro Asp Arg Phe Ser Gly Ser
50 55 60

Ser Ser Gly Asn Thr Ala Ser Leu Thr Ile Thr Gly Ala Gln Ala Glu
65 70 75 80

Asp Glu Ala Asp Tyr Tyr Cys Asn Ser Arg Asp Ser Ser Gly Asn His
85 90 95

Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly
100 105

<210> 17
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<213> Homo sapiens

<400> 17
Gln Ser Val Leu Thr Gln Pro Pro Ser Ala Ser Gly Thr Pro Gly Gln
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Arg Val Thr Ile Ser Cys Ser Gly Ser Ser Ser Asn Ile Gly Ser Asn
20 25 30

Tyr Val Tyr Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
35 40 45

Ile Tyr Arg Asn Asn Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
50 55 60

Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu Arg
65 70 75 80

Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Trp Asp Asp Ser Leu
85 90 95

Leu Ser Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly
100 105 110

<210> 18
<211> 108
<212> PRT

<213> Homo sapiens

<400> 18

Ser Ser Glu Leu Thr Gln Asp Pro Ala Val Ser Val Ala Leu Gly Gln
 1 5 10 15

Thr Val Arg Ile Thr Cys Gln Gly Asp Ser Leu Arg Ser Tyr Tyr Ala
 20 25 30

Ser Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Val Leu Val Ile Tyr
 35 40 45

Gly Lys Asn Asn Arg Pro Ser Gly Ile Pro Asp Arg Phe Ser Gly Ser
 50 55 60

Ser Ser Gly Asn Thr Ala Ser Leu Thr Ile Thr Gly Ala Gln Ala Glu
 65 70 75 80

Asp Glu Ala Asp Tyr Tyr Cys Asn Ser Arg Asp Ser Ser Gly Asn His
 85 90 95

Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly
 100 105

<210> 19

<211> 114

<212> PRT

<213> Homo sapiens

<400> 19

Asp Val Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro Gly
 1 5 10 15

Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Leu His Ser
 20 25 30

Asn Gly Tyr Asn Tyr Leu Asp Trp Tyr Leu Gln Lys Pro Gly Gln Ser
 35 40 45

Pro Gln Leu Leu Ile Tyr Leu Gly Ser Asn Arg Ala Ser Gly Val Pro
 50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
 65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln Ala
 85 90 95

Leu Gln Thr Pro Pro Phe Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile
 100 105 110

Lys Arg

<210> 20

<211> 112

<212> PRT

<213> Homo sapiens

<400> 20

Asp Ile Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro Gly
 1 5 10 15

Ala Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Leu His Ser
 20 25 30

Asn Gly Tyr Asn Tyr Leu Asp Trp Tyr Leu Gln Lys Pro Gly Gln Ser
 35 40 45

Pro Gln Leu Leu Ile Tyr Leu Gly Ser Asn Arg Ala Ser Gly Val Pro
 50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
 65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln Ala
 85 90 95

Leu Gln Thr Pro Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg
 100 105 110

<210> 21

<211> 112

<212> PRT

<213> Homo sapiens

<400> 21

Glu Ile Val Met Thr Gln Thr Pro Leu Ser Leu Ser Ile Thr Pro Gly
 1 5 10 15

Glu Gln Ala Ser Met Ser Cys Arg Ser Ser Gln Ser Leu Leu His Ser
 20 25 30
 Asp Gly Tyr Thr Tyr Leu Tyr Trp Phe Leu Gln Lys Ala Arg Pro Val
 35 40 45
 Ser Thr Leu Leu Ile Cys Glu Val Ser Asn Arg Phe Ser Gly Val Pro
 50 55 60
 Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
 65 70 75 80
 Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln Asp
 85 90 95
 Ala Gln Asp Pro Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg
 100 105 110

<210> 22
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 <212> PRT
 <213> Homo sapiens

<400> 22
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 1 5 10 15
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 20 25 30
 Gly Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Ser Gly Ile Asn Trp Asn Gly Gly Ser Thr Gly Tyr Ala Asp Ser Val
 50 55 60
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
 65 70 75 80
 Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95
 Ala Arg Leu Lys Met Pro His Ala Trp Gly Gln Gly Thr Leu Val Thr
 100 105 110

Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Ser
115 120 125

Ala Leu Ser Ser Glu Leu Thr Gln Asp Pro Ala Val Ser Val Ala Leu
130 135 140

Gly Gln Thr Val Arg Ile Thr Cys Gln Gly Asp Ser Leu Arg Ser Tyr
145 150 155 160

Tyr Ala Ser Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Val Leu Val
165 170 175

Ile Tyr Gly Lys Asn Asn Arg Pro Ser Gly Ile Pro Asp Arg Phe Ser
180 185 190

Gly Ser Ser Ser Gly Asn Thr Ala Ser Leu Thr Ile Thr Gly Ala Gln
195 200 205

Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Asn Ser Arg Asp Ser Ser Gly
210 215 220

Asn His Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly
225 230 235

<210> 23

<211> 242

<212> PRT

<213> Homo sapiens

<400> 23

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asn Ala
20 25 30

Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Gly Arg Ile Lys Ser Lys Thr Asp Gly Gly Thr Thr Asp Tyr Ala Ala
50 55 60 -

Pro Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr
65 70 75 80

Leu Tyr Leu Gln Met Asn Ser Leu Lys Thr Glu Asp Thr Ala Val Tyr

	85		90		95
Tyr Cys Ala Arg Asn Pro Lys Leu Val Lys Trp Gly Gln Gly Thr Leu					
	100		105		110
Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly					
	115		120		125
Gly Ser Ala Leu Gln Ser Val Leu Thr Gln Pro Pro Ser Ala Ser Gly					
	130		135		140
Thr Pro Gly Gln Arg Val Thr Ile Ser Cys Ser Gly Ser Ser Ser Asn					
	145		150		155
Ile Gly Ser Asn Tyr Val Tyr Trp Tyr Gln Gln Leu Pro Gly Thr Ala					
	165		170		175
Pro Lys Leu Leu Ile Tyr Arg Asn Asn Gln Arg Pro Ser Gly Val Pro					
	180		185		190
Asp Arg Phe Ser Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile					
	195		200		205
Ser Gly Leu Arg Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Trp					
	210		215		220
Asp Asp Ser Leu Leu Ser Val Phe Gly Gly Gly Thr Lys Leu Thr Val					
	225		230		235
					240
Leu Gly					

<210> 24
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 <213> Homo sapiens

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 20 25 30
 Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45

Ser Ala Ile Ser Gly Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
 50 55 60
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80
 Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95
 Ala Trp Lys Ser Leu Leu Met Leu Trp Gly Gln Gly Thr Leu Val Thr
 100 105 110
 Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Ser
 115 120 125
 Ala Leu Ser Ser Glu Leu Thr Gln Asp Pro Ala Val Ser Val Ala Leu
 130 135 140
 Gly Gln Thr Val Arg Ile Thr Cys Gln Gly Asp Ser Leu Arg Ser Tyr
 145 150 155 160
 Tyr Ala Ser Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Val Leu Val
 165 170 175
 Ile Tyr Gly Lys Asn Asn Arg Pro Ser Gly Ile Pro Asp Arg Phe Ser
 180 185 190
 Gly Ser Ser Ser Gly Asn Thr Ala Ser Leu Thr Ile Thr Gly Ala Gln
 195 200 205
 Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Asn Ser Arg Asp Ser Ser Gly
 210 215 220
 Asn His Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly
 225 230 235

<210> 25

<211> 131

<212> PRT

<213> Homo sapiens

<400> 25

Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Ser Ala
 1 5 10 15

Leu Asp Val Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro
 20 25 30

Gly Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Leu His
 35 40 45
 Ser Asn Gly Tyr Asn Tyr Leu Asp Trp Tyr Leu Gln Lys Pro Gly Gln
 50 55 60
 Ser Pro Gln Leu Leu Ile Tyr Leu Gly Ser Asn Arg Ala Ser Gly Val
 65 70 75 80
 Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys
 85 90 95
 Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln
 100 105 110
 Ala Leu Gln Thr Pro Pro Phe Thr Phe Gly Gln Gly Thr Lys Leu Glu
 115 120 125
 Ile Lys Arg
 130

<210> 26
 <211> 129
 <212> PRT
 <213> Homo sapiens

<400> 26
 Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Ser Ala
 1 5 10 15
 Leu Glu Ile Val Met Thr Gln Thr Pro Leu Ser Leu Ser Ile Thr Pro
 20 25 30
 Gly Glu Gln Ala Ser Met Ser Cys Arg Ser Ser Gln Ser Leu Leu His
 35 40 45
 Ser Asp Gly Tyr Thr Tyr Leu Tyr Trp Phe Leu Gln Lys Ala Arg Pro
 50 55 60
 Val Ser Thr Leu Leu Ile Cys Glu Val Ser Asn Arg Phe Ser Gly Val
 65 70 75 80
 Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys
 85 90 95
 Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln

100

105

110

Asp Ala Gln Asp Pro Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
 115 120 125

Arg

<210> 27
 <211> 98
 <212> PRT
 <213> Homo sapiens

<400> 27
 Glu Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Arg Pro Gly Gly
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asp Asp Tyr
 20 25 30

Gly Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45

Ser Gly Ile Asn Trp Asn Gly Gly Ser Thr Gly Tyr Ala Asp Ser Val
 50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
 65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Ala Arg

<210> 28
 <211> 98
 <212> PRT
 <213> Homo sapiens

<400> 28
 Glu Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Arg Pro Gly Gly
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asp Asp Tyr
 20 25 30

Gly Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45

Ser Gly Ile Asn Trp Asn Gly Gly Ser Thr Gly Tyr Ala Asp Ser Val
 50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
 65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Leu Tyr His Cys
 85 90 95

Ala Arg

<210> 29
 <211> 5
 <212> PRT
 <213> Homo sapiens

<400> 29
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<210> 30
 <211> 17
 <212> PRT
 <213> Homo sapiens

<400> 30
 Gly Ile Asn Trp Asn Gly Gly Ser Thr Gly Tyr Ala Asp Ser Val Lys
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Gly

<210> 31
 <211> 100
 <212> PRT
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<400> 31
 Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asn Ala
20 25 30

Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Gly Arg Ile Lys Ser Lys Thr Asp Gly Gly Thr Thr Asp Tyr Ala Ala
50 55 60

Pro Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr
65 70 75 80

Leu Tyr Leu Gln Met Asn Ser Leu Lys Thr Glu Asp Thr Ala Val Tyr
85 90 95

Tyr Cys Ala Arg
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<313> 32
<211> 100
<212> PRT
<213> Homo sapiens

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Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asn Ala
20 25 30

Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Gly Arg Ile Lys Ser Lys Thr Asp Gly Gly Thr Thr Asp Tyr Ala Ala
50 55 60

Pro Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr
65 70 75 80

Leu Tyr Leu Gln Met Asn Ser Leu Lys Thr Glu Asp Thr Ala Val Tyr
85 90 95

Tyr Cys Thr Thr
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 <211> 5
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 Arg Ile Lys Ser Lys Thr Asp Gly Gly Thr Thr Asp Tyr Ala Ala Pro
 1 5 10 15

Val Lys Gly

<210> 35
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 <212> PRT
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<400> 35
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 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30

Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45

Ser Ala Ile Ser Gly Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
 50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Ala Trp

<210> 36
 <211> 98
 <212> PRT
 <213> Homo sapiens

<400> 36
 Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
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 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30
 Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Ser Ala Ile Ser Gly Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
 50 55 60
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80
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 85 90 95
 Ala Arg

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<400> 38
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1 5 10 15

Gly

<210> 39
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<400> 39
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<210> 40
 <211> 6
 <212> PRT
 <213> Homo sapiens

<400> 40
 Asn Pro Lys Leu Val Lys
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<210> 41
 <211> 6
 <212> PRT
 <213> Homo sapiens

<400> 41
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 1 5

<210> 42
 <211> 96
 <212> PRT
 <213> Homo sapiens

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 1 5 10 15

Thr Val Arg Ile Thr Cys Gln Gly Asp Ser Leu Arg Ser Tyr Tyr Ala
 20 25 30

Ser Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Val Leu Val Ile Tyr
 35 40 45
 Gly Lys Asn Asn Arg Pro Ser Gly Ile Pro Asp Arg Phe Ser Gly Ser
 50 55 60
 Ser Ser Gly Asn Thr Ala Ser Leu Thr Ile Thr Gly Ala Gln Ala Glu
 65 70 75 80
 Asp Glu Ala Asp Tyr Tyr Cys Asn Ser Arg Asp Ser Ser Gly Asn His
 85 90 95

<210> 43
 <211> 11
 <212> PRT
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<400> 43
 Gln Gly Asp Ser Leu Arg Ser Tyr Tyr Ala Ser
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<210> 44
 <211> 7
 <212> PRT
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 Gly Lys Asn Asn Arg Pro Ser
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<210> 45
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 <212> PRT
 <213> Homo sapiens

<400> 45
 Asn Ser Arg Asp Ser Ser Gly Asn His
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<210> 46
 <211> 98

<212> PRT

<213> Homo sapiens

<400> 46

Gln Ser Val Leu Thr Gln Pro Pro Ser Ala Ser Gly Thr Pro Gly Gln
 1 5 10 15

Arg Val Thr Ile Ser Cys Ser Gly Ser Ser Ser Asn Ile Gly Ser Asn
 20 25 30

Tyr Val Tyr Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
 35 40 45

Ile Tyr Arg Asn Asn Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
 50 55 60

Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu Arg
 65 70 75 80

Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Trp Asp Asp Ser Leu
 85 90 95

Leu Ser

<210> 47

<211> 98

<212> PRT

<213> Homo sapiens

<400> 47

Gln Ser Val Leu Thr Gln Pro Pro Ser Ala Ser Gly Thr Pro Gly Gln
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Arg Val Thr Ile Ser Cys Ser Gly Ser Ser Ser Asn Ile Gly Ser Asn
 20 25 30

Tyr Val Tyr Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
 35 40 45

Ile Tyr Arg Asn Asn Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
 50 55 60

Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu Arg
 65 70 75 80

Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Trp Asp Asp Ser Leu

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90

95

Ser Gly

<210> 48

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Ser Gly Ser Ser Ser Asn Ile Gly Ser Asn Tyr Val Tyr

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<213> Homo sapiens

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5

<210> 51

<211> 96

<212> PRT

<213> Homo sapiens

<400> 51

Ser Ser Glu Leu Thr Gln Asp Pro Ala Val Ser Val Ala Leu Gly Gln

1

5

10

15

Thr Val Arg Ile Thr Cys Gln Gly Asp Ser Leu Arg Ser Tyr Tyr Ala

20

25

30

Ser Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Val Leu Val Ile Tyr
 35 40 45
 Gly Lys Asn Asn Arg Pro Ser Gly Ile Pro Asp Arg Phe Ser Gly Ser
 50 55 60
 Ser Ser Gly Asn Thr Ala Ser Leu Thr Ile Thr Gly Ala Gln Ala Glu
 65 70 75 80
 Asp Glu Ala Asp Tyr Tyr Cys Asn Ser Arg Asp Ser Ser Gly Asn His
 85 90 95

<210> 52
 <211> 11
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<400> 52
 Gln Gly Asp Ser Leu Arg Ser Tyr Tyr Ala Ser
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<210> 53
 <211> 7
 <212> PRT
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<400> 53
 Gly Lys Asn Asn Arg Pro Ser
 1 5

<210> 54
 <211> 9
 <212> PRT
 <213> Homo sapiens

<400> 54
 Asn Ser Arg Asp Ser Ser Gly Asn His
 1 5

<210> 55
 <211> 16

<212> PRT

<213> Homo sapiens

<400> 55

Arg Ser Ser Gln Ser Leu Leu His Ser Asn Gly Tyr Asn Tyr Leu Asp
 1 5 10 15

<210> 56

<211> 7

<212> PRT

<213> Homo sapiens

<400> 56

Leu Gly Ser Asn Arg Ala Ser
 1 5

<210> 57

<211> 9

<212> PRT

<213> Homo sapiens

<400> 57

Met Gln Ala Leu Gln Thr Pro Pro Phe
 1 5

<210> 58

<211> 7

<212> PRT

<213> Homo sapiens

<400> 58

Met Gln Ala Leu Gln Thr Pro
 1 5

<210> 59

<211> 102

<212> PRT

<213> Homo sapiens

<400> 59

Asp Val Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro Gly
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Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Leu His Ser

	20		25		30
Asn Gly Tyr Asn Tyr Leu Asp Trp Tyr Leu Gln Lys Pro Gly Gln Ser					
35		40		45	
Pro Gln Leu Leu Ile Tyr Leu Gly Ser Asn Arg Ala Ser Gly Val Pro					
50		55		60	
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile					
65		70		75	80
Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln Ala					
	85		90		95
Leu Gln Thr Pro Pro Phe					
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 <213> Homo sapiens

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1 5 10 15
Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Leu His Ser
20 25 30
Asn Gly Tyr Asn Tyr Leu Asp Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45
Pro Gln Leu Leu Ile Tyr Leu Gly Ser Asn Arg Ala Ser Gly Val Pro
50 55 60
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80
Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln Ala
85 90 95
Leu Gln Thr Pro
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<210> 61
 <211> 100

<212> PRT

<213> Homo sapiens

<400> 61

Glu Ile Val Met Thr Gln Thr Pro Leu Ser Leu Ser Ile Thr Pro Gly
1 5 10 15

Glu Gln Ala Ser Met Ser Cys Arg Ser Ser Gln Ser Leu Leu His Ser
20 25 30

Asp Gly Tyr Thr Tyr Leu Tyr Trp Phe Leu Gln Lys Ala Arg Pro Val
35 40 45

Ser Thr Leu Leu Ile Cys Glu Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln Asp
85 90 95

Ala Gln Asp Pro
100

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/25495

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : Please See Extra Sheet

US CL : Please See Extra Sheet

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 436/548; 435/7.1, 320.1, 326, 328; 514/12; 424/139.1; 530/387.1, 388.22; 536/23.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
patents, embase, biosis, medline, caplus

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	GRIFFITHS et al. Isolation of high affinity human antibodies directly from large synthetic repertoires. EMBO. J. 1994, Vol. 13, No. 14, pages 3245-3260, especially pages 3245, 3254-59.	1-5
Y	NISSIM et al. Antibody fragments from a `single pot` phage display library as immunochemical reagents. EMBO. J. 1994, Vol 13, No. 3, pages 692-698, especially pages 692 and 696-698.	1-5
Y	Miller et al. Mimotope/anti-mimotope probing of structural relationships in platelet glycoprotein Iba Proc. Natl. Acad. Sci., USA. April 1996, Vol. 93, pages 3565-3569, especially page 3565.	7, 12, 17, 22-27, 30, 33, 38

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

A	Special categories of cited documents: document defining the general state of the art which is not considered to be of particular relevance	*T*	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
B	earlier document published on or after the international filing date	*X*	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
L	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*Y*	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
O	document referring to an oral disclosure, use, exhibition or other means	*Z*	document member of the same patent family
P	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

16 JANUARY 2000

Date of mailing of the international search report

10 FEB 2000

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorized officer

AMY DECLoux

Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/25495

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	KONKLE et al. Cytikine-enhanced Expression of Glycoprotein Iba in Human Endothelium. The Journal of Biological Chemistry. 1990, Vol. 265, No. 32, pages 19833-19838, especially pages 19833-98834.	7, 12, 17, 22-27, 30, 33 and 38.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/25495

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 6, 8-11, 13-16, 18-21, 28-29, 31-32, 34-37, 39-52
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

No CRF for this case has been filed. The instant claims recite SEQ ID NO:s or depend therefrom and cannot be searched other than a sequence search.

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/25495

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (7):

G01N 33/53; A61K 38/02, 39/395; C07K 14/435, 16/28; C12N 15/63, 15/66, 15/85, 15/86, 15/11

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

436/548; 435/7.1, 320.1, 326, 328; 514/12; 424/139.1; 530/387.1, 388.22; 536/23.1

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